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Title of Dissertation: "Impulsive Action, Psychological Stress, and Behavioral Sensitization to Nicotine in a Rat Model of Impulsivity"

Name of Candidate: Kristen R. Hamilton
Doctor of Philosophy Degree
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## ABSTRACT

Title of Thesis: Impulsive Action, Psychological Stress, and Behavioral Sensitization to Nicotine in a Rat Model of Impulsivity

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Thesis directed by: Neil E. Grunberg, Ph.D., Professor
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Impulsivity, a construct characterized by immediate action without consideration of future consequences, is associated with cigarette smoking. Psychological stress increases cigarette smoking, and this effect may be augmented in impulsive individuals. Increased nicotine reinforcement under stress and in impulsive individuals may underlie relationships among the variables. The effect of stress on impulsive action and reinforcing actions of nicotine, and whether such effects differ in impulsive and non-impulsive individuals, is not known. The present research examined effects of stress on impulsive action, attention, and nicotine behavioral sensitization in Lewis (impulsive) and Fischer (non-impulsive) rats. Subjects were 32 male Lewis and Fischer rats used in a 2 (Lewis, Fischer) x 2 (stress, non-stress) factorial design with repeated measures. The research was divided into two conceptually distinct experiments using the same subjects. In Experiment 1, rats' impulsive action and attention were measured in the Five Choice Serial Reaction Time Task (5-CSRTT), and their locomotor activity was measured in locomotor activity chambers. In Experiment 2, rats' locomotor activity was measured daily immediately after they received injections of 0.5 mg/kg nicotine. Rats also were tested in the 5-CSRTT in Experiment 2, after locomotor activity was measured. Rats' serum corticosterone was measured

after the conclusion of the Experiment 2. Several conclusions can be drawn from the present research: (1) Lewis and Fischer rats provide a valid rat model of impulsivity, with Lewis rats as the more impulsive rat strain. (2) Stress had an effect on impulsivity. Stress affected impulsivity differentially in Lewis (impulsive) and Fischer (non-impulsive) rats—stress decreased impulsivity in Lewis rats and increased impulsivity in Fischer rats. (3) Stress decreased attention. (4) Reinforcing actions of nicotine were greater in impulsive than non-impulsive organisms. (5) Stress increased nicotine reinforcement in impulsive organisms and decreased nicotine reinforcement in non-impulsive organisms. The present results are relevant to understanding why cigarette smoking is increased in impulsive and stressed individuals. Stress may increase cigarette smoking by making nicotine more reinforcing for impulsive individuals and by increasing impulsivity in non-impulsive individuals.

# Impulsive Action, Psychological Stress, and Behavioral Sensitization to Nicotine in a Rat Model of Impulsivity

by

Kristen R. Hamilton

Doctoral Dissertation submitted to the Faculty of the Department of Medical and Clinical Psychology Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 2010

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things go well. I have always trusted and counted on Amy, and she has never let me down. Amy is like a sister to me.

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# INTRODUCTION

## Overview

The psychological construct impulsivity is a tendency to act immediately without regard for future consequences (Moeller, Barratt, Dougherty, Schmitz, & Swann, 2001). Impulsivity is correlated with a variety of detrimental behaviors, including cigarette smoking. Impulsivity increases likelihood of engaging in cigarette smoking in humans (e.g., Mitchell, 1999) and nicotine self-administration in animal models (e.g., Diergaarde, Pattij, Poortvliet, Hogenboom, DeVries, Schoffelmeer, & DeVries, 2008). Psychological stress also is associated with the initiation and maintenance of tobacco use (Kassel, Stroud, & Patronis, 2003) and other drug use (e.g., Grunberg, Berger, & Hamilton, 2010; Goeders, 2002; Shaham & Stewart, 1995; Shaham, Shalev, Lu, De Wit, & Stewart, 2003; Hilakivi-Clarke & Lister, 1992). Although impulsivity and stress each is relevant to drug use, the relationship between stress and impulsivity has received little research attention. Psychological stress may increase state impulsivity, and this effect may be greater in impulsive individuals. Further, reinforcing actions of nicotine may be increased in individuals with trait impulsivity, and may be differentially altered by stress. The present research examined effects of stress on impulsive action and nicotine reinforcement in a rat model of impulsivity. The specific aims of this work were to determine, using a rat model of impulsivity, whether stress: (1) increased impulsive action, (2) increased reinforcing actions of nicotine, and (3) had different effects in impulsive individuals compared with non-impulsive individuals.

The research literature on impulsivity, stress, and nicotine is reviewed in the present paper and the links between impulsivity and nicotine use and stress and nicotine use are discussed. Following from this background, arguments are made for an effect of stress on impulsive action and reinforcing actions of nicotine. Two experiments using an animal model of impulsivity were designed to address these research questions. The first experiment examined effects of stress on impulsive action and attention and the second experiment examined effects of stress on reinforcing actions of nicotine in Lewis (impulsive) and Fischer (non-impulsive) rats. Use of an animal model in the present research allowed for true experimental control. Methodology, results, and discussion for each of the two experiments are presented, followed by a general discussion about effects of stress on impulsive action and nicotine reinforcement.

## Impulsivity

Impulsivity is a tendency toward immediate action without consideration of future consequences (Moeller et al., 2001). Impulsive individuals react rapidly and without forethought to external stimuli, such as environmental events and others' actions, and to internal stimuli, such as ideas and emotions (Moeller et al., 2001). The consequences impulsive individuals fail to consider when executing poorly planned actions can include risk and injury to themselves and others, strained social relationships, communicable diseases, and financial misfortunes. Because impulsivity is a characteristic pattern of behavior and responding to the world, it can be considered a psychological trait, as

conceptualized by Barratt (1959; Patton, Stanford, & Barratt, 1995) and others. However, because impulsivity varies in different situations, it can also be considered a psychological state (Evenden, 1999; Dougherty, Mathias, Marsh, & Jagar, 2005). The consequences of impulsive actions (e.g.,health risk behavior such as tobacco use) can be devastating to individuals and to society—for this reason it is important to understand factors that influence state impulsivity in people with and without trait impulsivity.

### *Trait Impulsivity*

Trait impulsivity represents a stable personality characteristic in humans that also can be modeled in animals. Human measures of trait impulsivity include Barratt's Impulsiveness Scale (BIS-11) (Patton et al., 1995) and Eysenck's Personality Questionnaire (Eysensck & Eysenck, 1975). The BIS-11 is a 30 item questionnaire that requires participants to characterize their usual behavior on three dimensions of impulsivity: cognitive, motor, and non-planning (Patton et al., 1995). Eysenck's Personality Questionnaire is divided into three dimensions of personality: Extraversion, Neuroticism, and Psychoticism. Impulsivity is a component of the psychoticism dimension of personality (Eysenck & Eysenck, 1985). The conceptualization of impulsivity as a personality trait implies that it represents a stable pattern of behavior and cognition. People with high trait impulsivity tend to act impulsively across all situations. In rats, stable rat strain differences in impulsivity can be used to model trait impulsivity. In the present research, Lewis and Fischer rats model differences in trait impulsivity.

The Lewis rat strain has consistently performed more impulsively than Fischer rats on behavioral measures of impulsivity in previous research (e.g., Anderson & Woolverton, 2005; Kearns, Gomez-Serrano, Weiss, & Riley, 2006).

### *State impulsivity*

While impulsivity may be a stable personality trait, it also can be conceptualized as a psychological state that fluctuates over time. State impulsivity is measured by behavioral tasks that index an individual's level of impulsivity at a given moment in humans (Dougherty et al., 2005) or rats (e.g., Robbins, 2002). Manifestations of state impulsivity can be classified into two distinct types: impulsive action (behavioral disinhibition) and impulsive choice (impulsive decision making) (Winstanley, Dalley, Theobald, & Robbins, 2004a; Winstanley, Eagle, & Robbins, 2006; Winstanley, Theobald, Dalley, Glennon, & Robbins, 2004b). Impulsive action reflects a deficit in inhibitory control (Winstanley et al., 2006; Diergaarde et al., 2008) while impulsive choice reflects decision-making processes in which individuals cannot tolerate delay (Winstanley et al., 2006; Diergaarde et al., 2008). The two types of impulsivity are not always correlated in an individual (Winstanley et al., 2004), and are mediated by different neurobiological mechanisms (vanGaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006; vanGaalen, vanKoten, Schoffelmeer, & Vanderschuren, 2006). Impulsive action was examined in the present experiments because it predicted initiation and maintenance of nicotine self-administration in a rat model (Diergaarde et al., 2008). Impulsive choice, which predicted nicotine seeking

during abstinence and nicotine reinstatement after exposure to cues in a rat model (Diergaarde et al., 2008), is more relevant to behavior after nicotine addiction has been established. Because the present research was concerned with factors relevant to the initiation and maintenance of tobacco use, impulsive action was examined rather than impulsive choice.

Impulsive action is behavioral disinhibition, or an inability to withhold a response (Winstanley et al., 2006). Impulse control can be conceptualized as a mechanism that inhibits pre-potent desires for reward to allow more planful and strategic cognitive processes to occur (Winstanley et al., 2006). Impulsive action is a deficit in inhibitory control, and reflects a motoric component of impulsivity. Impulsive action is measured in humans and animals by tasks that require animals to inihibit a response until an appropriate time, such as the Five-Choice-Serial-Reaction-Time task (5-CSRTT) or the Go/No–Go Task (Winstanley et al., 2006). In the 5-CSRTT, rats are required to withhold from responding until the appropriate time, with premature responding providing an index of impulsive action. In the present research, effects of stress on impulsive action, as measured by the 5-CSRTT, were measured.

### *Measuring Impulsivity*

Impulsivity can be measured in humans and animals using a variety of behavioral tasks. Behavioral assays of impulsivity in humans and animals are often designed to be parallel to one another (Chudasama & Robbins, 2006) and many tasks for animals were developed as analogues for tasks used in humans

(e.g., the 5-CSRTT was modeled after the Continuous Performance Task). There are two major categories of impulsivity measures: those that measure impulsive action and those that measure impulsive choice. Impulsive action is measured by tasks that require subjects to inhibit a behavioral response until the appropriate signal is given. Tasks that measure impulsive action include the continuous performance task (CPT) for humans (e.g., Rosvold, Mirsky, Sarason, Bransome, & Beck, 1956), and the Go/No-go task (e.g., Logue, Swartz, & Wehner, 1998) and the 5-CSRTT for rodents (Robbins, 2002). Impulsive action is measured in the present experiments because of its role in initiation of nicotine self-administration (e.g., Diergaarde et al., 2008). Impulsive choice is measured by tasks that require the participant to choose between a larger delayed reward and a smaller, immediate reward. Tasks that measure impulsive choice include the Iowa Gambling task (e.g., Bechara et al., 1994) and the Cambridge Gamble task for humans (Clark, Cools, & Robbins, 2004) and the delayed reward task for rodents (e.g., Winstanley et al., 2004a). Because impulsive action (which is relevant to drug use) is measured in the present research, features of behavioral assays that measure impulsive action are discussed below.

### *Cognitive Variables in Impulsive Action*

The Continuous Performance Test (CPT) measures sustained and selective attention and impulsivity exclusively in humans (e.g., Rosvold et al., 1956). In the CPT, participants are required to respond to a stimulus quickly, but only when it is preceded by a specific stimulus. Errors of commission that occur

with fast reaction times reflect impulsivity, whereas errors of commission that occur with slow reaction times reflect inattention (Halperin et al., 1988).

The 5-CSRTT is a rodent analogue of the CPT (Robbins, 2002), and measures aspects of attention and impulsivity. In the 5-CSRTT, rats are required to sustain and divide attention among five spatial locations to detect a brief illumination of a cue-light. Animals are required to make a response in the nose-poke aperture in which the visual stimulus occurred immediately after detection of the stimulus. The task requires subjects to withhold from making a response until presentation of the visual stimulus; premature responses reflect impulsivity. Unlike in the Go/No-go task, successful response inhibition is not reinforced in the 5-CSRTT. Mitchell (2004) suggests that cognitive processes underlying premature responding may differ somewhat from those underlying reinforced inhibition, with reinforced inhibition requiring more complex attentional processes. Use of the 5-CSRTT in the present research allowed for assessment of impulsivity without the increased cognitive load that may be associated with the Go/No-go task.

### *Neurobiological Mechanisms of Impulsive Action*

The neurobiology of impulsivity involves interactions among multiple neurotransmitter systems, neural structures, and neural circuits (e.g., Pattij & Vanderschuren, 2008; Muir, Everitt, & Robbins, 1996). Impulsive behavior is the manifestation of an imbalance between systems that subserve inhibition and activation. Understanding the neurobiology of impulsive action is useful to

understand impulsivity and to generate specific hypotheses about impulsivity, stress, and actions of nicotine.

Response inhibition requires interplay of many neural structures and neurotransmitters. The prefrontal cortex is important for higher-level executive functions, with the right inferior frontal cortex (IFC) playing a critical role in response inhibition, as revealed by multiple brain-imaging and lesion studies (Aron & Poldrack, 2005). The role of the right IFC in response inhibition may depend partially on noradrenergic modulation from the brainstem locus coeruleus (LC) (Aron & Poldrack, 2005), a structure that interacts with the lateral prefrontal cortex (lPFC). Lesion studies with rodents reveal specific areas of the medial prefrontal cortex (mPFC) that mediate distinct aspects of response inhibition (Chudasama, Passetti, Desai, Rhodes, Lopian, & Robbins, 2004; Chudasama & Muir, 2001; Dalley, Cardinal, & Robbins, 2004). Glutamate neurotransmission in the infralimbic cortex (IL) of the medial prefrontal cortex is important for response inhibition and control of premature responding (Murphy, Dalley, & Robbins, 2005). An index of impulsive action, premature responding in the 5-CSRTT, results from deficits in the IL cortex (Chudasama et al., 2004). Response inhibition involves the post-genual anterior cingulate cortex of the medial prefrontal cortex, and response disinhibition often results from deficits in this area (Muir, et al., 1996). Perseveration, which is also related to impulsive action, results from deficits in the orbitofrontal cortex (OFC) (Chudasama & Muir, 2001). There also may be a role for the striatum in response inhibition, although

evidence is mixed (Aron & Poldrack, 2005). Response inhibition was examined behaviorally in the present research using the 5-CSRTT.

Many neurotransmitters, including glutamate, serotonin, noradrenaline, and dopamine, are implicated in response inhibition. Global serotonin neurotransmission is important for response inhibition (Chudasama & Robbins, 2006). Noradrenaline and dopamine also are implicated in response inhibition, and their tonic and phasic modes may be relevant for different aspects of cognitive function (Aron & Poldrack, 2005). Noradrenaline neurotransmission may subserve vigilance and other cognitive processes important for response inhibition (Arnsten & Li, 2005). The interaction among many neural structures and neurotransmitters is required for successful response inhibition, and abnormalities in any of these components can lead to disinhibition.

Contrasting with the inhibitory system, the nucleus accumbens of the ventral striatum is a limbic structure involved in the invigoration of impulsive acts. In particular, increased dopamine (DA) neurotransmission in this area leads to increased impulsive action (Pattij & Vanderschuren, 2008; Robbins, 2002; van Gaalen et al., 2006). The two rat strains used in the present research as a rat model of impulsivity differ in DA neurotransmission, with Lewis (impulsive) rats having greater DA neurotransmission than Fischer (non-impulsive) rats. Because psychological stress and acute nicotine administration increase DA neurotransmission, it was hypothesized in the present research that stress and nicotine administration would increase impulsive action in Lewis and Fischer rats, with a greater effect in the Lewis rat strain.

The multiple neurotransmitter systems that influence impulsivity include serotonin, noradrenaline, glutamate, and dopamine (Pattij & Vanderschuren, 2008). Altered serotonin (5-HT) neurotransmission is implicated in impulsivity, but the exact mechanisms by which 5-HT neurotransmission impact impulsivity are unclear (Pattij & Vanderschuren, 2008). A large amount of empirical evidence supports the hypothesis that decreased 5-HT neurotransmission correlates with disinhibition (Soubrie et al., 1986; Masaki et al., 2006; Winstanley et al., 2004a; Winstanley et al., 2004b, Chudasama and Robbins, 2006), although some investigations do not support this conclusion (Dalley, Theobald, Eagle, Passetti, & Robbins, 2002). The complexity of the 5-HT system and its interactions with other neurotransmitters that also influence impulsivity, such as noradrenaline, glutamate, and DA, are factors that make the role of serotonin in impulsivity difficult to ascertain (Pattij & Vanderschuren, 2008). Increased noradrenaline neurotransmission decreases impulsive action (van Gaalen et al., 2006; Paine, Tomasiewicz, Zhang, & Carlezon, 2007; Robinson, et al., 2008). Several research lines indicate that decreased glutamate neurotransmission increases impulsive action (Mirjana, Baviera, Invernizzi, & Balducci, 2004; Higgins, Ballard, Huwyler, Kemp, & Gill, 2003), particularly when glutamate neurotransmission is decreased in the medial prefrontal cortex (Mirjana et al., 2004; Mirjana, Baviera, Invernizzi, & Balducci, 2006; Murphy et al., 2005).

The role of dopamine neurotransmission in impulsivity is well-established (Pattij & Vanderschuren, 2008; Robbins, 2002; van Gaalen et al., 2006). While DA neurotransmission will not be examined in the proposed experiments, an

understanding of DA neurotransmission patterns in impulsive action can guide the development of specific hypotheses about impulsivity, stress, and actions of nicotine. Factors affecting DA neurotransmission include DA release from axon terminals and availability of DA in the synapse (Michael & Borland, 2006). DA transporters (DAT) are important regulators of DA neurotransmission because they clear DA from the extracellular space after release, thereby terminating the DA signal (Michael & Borland, 2006). Genetic polymorphisms resulting in relatively increased striatal DA release and synaptic availability and decreased postsynaptic inhibition are associated with relatively greater reward-related ventral striatum reactivity, which covaries with impulsivity (Forbes, Brown, Kimak, Ferrell, Manuck, & Hariri, 2009).

Rats that were used as a model of impulsivity in the present research, Lewis rats, have increased dopamine neurotransmission compared with Fischer rats (Kosten & Ambrosio, 2002). Specifically, Lewis rats have lower levels of DA transporters in the nucleus accumbens (Flores, Wood, Barbeau, Quirion, & Srivastava, 1998) and prolonged elevation of DA levels in the synapse after cocaine and methamphetamine administration compared with Fischer rats (Camp, Browman, & Robinson, 1994; Strecker, Eberle, & Ashby, 1995). The higher levels of dopamine neurotransmission in Lewis rats compared to Fischer rats will lead to increased impulsivity and increased nicotine reinforcement in the Lewis rats in the present research.

Increased DA neurotransmission underlies impulsive action (van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006b).

Pharmacological manipulations that increased DA neurotransmission increased impulsive action and manipulations that decreased DA neurotransmission decreased impulsive action (van Gaalen et al., 2006b). It follows from these reports that increasing DA neurotransmission (i.e., psychostimulant administration, drug cues, stress) will increase impulsive action. For this reason, it was hypothesized that stress and nicotine administration would increase impulsive action.

### *Impulsivity and Attention*

Attention involves the allocation of mental resources to pertinent stimuli to enable the mental processing of the stimuli (James, 1890). Attentional deficits and impulsive actions may occur together (Barkley, 1997). Attentional control and impulse control are two distinct but related constructs required for the execution of an appropriate action. As an individual is deciding upon an action, he or she must consider all possible behavioral choices and their consequences and select the most appropriate action—a process requiring both attentional control and impulse control. Attention is required for the mental processing of all possible behavioral choices and their consequences. Impulse control is required for the inhibition of actions while all possible behavioral choices are considered. A failure of either impulse control or attention can result in the execution of an inappropriate act. Impulsivity and attentional deficits are both components of Attention-Deficit Hyperactivity Disorder (ADHD). Barkley (1997) proposed that impulsivity and attention are related and unified components of ADHD.

Consistent with the conceptualization of attention and impulsivity as related constructs, both attention and impulsivity are measured in the 5-CSRTT. Sustained attention was measured in the 5-CSRTT in the present research. Sustained attention, or vigilance, is the continuous allocation of processing resources for the detection of rare events (Robbins, 2002). Just as Lewis and Fischer rats have not been compared on measures of impulsive action, they also have not been compared on measures of attention. Whether Lewis and Fischer rats differ in attention is not known. In the present research, Lewis and Fischer rats will be compared on measures of attention as well as measures of impulsive action.

### *Impulsivity and Health Risk Behaviors*

The importance of understanding factors that affect impulsivity is underscored by the well-established role of impulsivity in health risk behaviors. Impulsivity is implicated in a variety of detrimental human behaviors, including drug abuse, violence, risky sexual behavior, and suicide (e.g., Barratt, Stanford, Dowdy, Liebman, & Kent, 1999; McCoul & Haslam, 2001; Perry & Carroll, 2008; Beautrais, Joyce, & Mulder, 1999). For example, impulsivity is associated with tobacco use (e.g., Mitchell, 1999), pathological gambling (e.g., Blanco, Potenza, Kim, Ibáñez, Zaninelli, Saiz-Ruiz, & Grant, 2009) and disordered eating, such as binge-eating (e.g., Dawe & Loxton, 2004; Yeomans, Leitch, & Mobini, 2008). Impulsivity is implicated in suicide attempts and completions (e.g., Swann, Dougherty, Pazzaglia, Pham, Steinberg, & Moeller, 2005; Zouk, Tousignant,

Seguin, Lesage, & Turecki, 2006). Impulsivity is associated with high risk sexual behavior and contraction of multiple sexually transmitted infections (e.g., Kalichman & Cain, 2004; McCoul & Haslam, 2001). Additionally, there is a role of impulsivity in aggression and violence toward others (Barratt, Stanford, Dowdy, Liebman, & Kent, 1999; Edwards, Scott, Yarvis, Paizis, & Panizzon, 2008). Further, there is considerable overlap among health risk behaviors, with impulsivity acting as the common denominator among the behaviors (e.g., Wolf & Maisto, 2000; Holderness, Brooks-Gunn, & Warren, 1994; Culbert & Klump, 2005; Schafer, Blanchard, & Fals-Stewart, 1994).

## Drug Use

Impulsivity enhances vulnerability to drug abuse (i.e., Perry & Carroll, 2008). Neurobiological, psychological, and behavioral changes occur during the transition from drug use to dependence (Koob et al., 2004). Koob and colleagues (2004) characterize the addiction cycle as a progression from impulsivity to compulsivity. Three distinct stages occur during the progression from drug use to drug dependence: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (Koob et al., 2004). The stages of addiction can be modeled in experimental animals (Sanchis-Segura & Spanagel, 2006), which allows for the determination of causal factors in the transition from drug use to drug dependence. Factors relevant to initiation and maintenance of tobacco use were examined in the present research because the phase is an important target for prevention and treatment strategies.

*Impulsivity and Drug Use*

Impulsivity influences progression through the addiction cycle, and relations between impulsivity and addiction stages have been examined using animal models (Perry & Carroll, 2008). Nicotine is the addictive drug in tobacco that maintains smoking behavior (e.g., United States Department of Health and Human Services, 1988; Grunberg, Faraday, & Rahman, 2000). Nicotine reinforcement, which was examined in the present research, may influence progression through the nicotine addiction cycle. Nicotine reinforcement refers to a pleasurable, positively reinforcing state induced by drug use (e.g., Koob & LeMoal, 1997; Robinson & Berridge, 2003). Progression through the addiction cycle is generally established by allowing animals extended access to a drug of abuse, such as nicotine or cocaine, and monitoring self-administration to identify the emergence of addiction-like behaviors that signal the transition to drug dependence (Koob et al., 2004; Diergaarde et al., 2008; Perry & Carroll, 2008).

In rats, impulsive action predicts acquisition of nicotine self-administration (Diergaarde et al., 2008) and cocaine self-administration (Dalley et al., 2007), results that are consistent with clinical research of impulsive action in drug abusers (Perry and Carroll, 2008; Spinella, 2002, Fillmore & Rush, 2002; Li, Milivojevic, Kemp, Hong, & Sinha, 2006; Monterosso, Aron, Cordova, Xu, & London, 2005). Additionally, impulsivity is associated with cue-induced reinstatement of nicotine-seeking and drug-induced reinstatement of cocaine-seeking in animal models (Perry et al., 2008; Diergaarde et al., 2008), results that also are consistent with clinical research examining trait impulsivity (Doran et al.,

2004; 2007) (See Table 1 on page 20). Using a protracted access paradigm, Belin, Mar, Dalley, Robbins, and Everitt (2008) revealed the role of impulsivity in the progression of the addiction cycle; high impulsive action predicts the transition from controlled to compulsive-cocaine taking. These results emphasize how impulsive action increases an individual's vulnerability to developing drug addiction.

Perry and Carroll (2008) thoroughly reviewed the literature on drug use and impulsivity and consider the role of impulsivity in drug use. These authors proposed three non-mutually exclusive hypotheses about the role of impulsivity in drug use: (1) increased levels of impulsivity lead to drug abuse; (2) drugs of abuse increase impulsivity; (3) impulsivity and drug use are associated through a common third factor. The present experiments are relevant to Perry and Carroll's hypotheses.

*Impulsivity and tobacco use*

Studies of the impact of impulsivity on cigarette smoking are important because of tobacco's vast societal impact. Cigarette smoking is the leading cause of preventable death in the United States, and leads to significant health consequences, including cardiovascular diseases, cancers, and respiratory diseases (Centers for Disease Control [CDC], 2004). Despite these health consequences, nearly one-fifth of women and high school students, and one-quarter of men smoke cigarettes (CDC, 2009). People continue to smoke cigarettes largely because of nicotine, a highly addictive drug that plays a major

role in reinforcing the maintenance of tobacco use (Grunberg et al., 2000; Grunberg & Starosciak, 2010; Henningfield & Benowitz, 1995; Koob & LeMoal, 2008; USDHHS, 1988). Nicotine is the addictive psychoactive chemical found in all tobacco products that is largely responsible for tobacco smoking maintenance. The study of factors that affect nicotine addiction, such as impulsivity and stress, is important for treatment and prevention efforts.

Cigarette smokers are more impulsive than non-cigarette smokers on measures of trait and state impulsivity (Mitchell, 1999). Smokers and non-smokers were assessed for trait impulsivity on five personality questionnaires. State impulsivity was measured by performance on three behavioral choice tasks. On all measures, smokers were more impulsive than non-smokers, suggesting that impulsivity increases vulnerability to cigarette addiction. It is possible that increased nicotine reinforcement in impulsive individuals, which was examined in the present research, contributes to this relationship. Bickel and colleagues (1999) reported that current smokers performed more impulsively than ex-smokers and those who have never smoked on a behavioral measure of impulsivity. The authors suggested that these results indicate that cigarette smoking increases impulsivity and that this effect is reversible, because ex-smokers no longer had increased levels of impulsivity. This effect would support Perry and Carroll's second hypothesis that drugs of abuse increase impulsivity. However, alternative explanations for the results of Bickel et al. (1999) and Mitchell (1999) cannot be ruled out because of the quasi-experimental design. For example, the lower level of impulsivity in ex-smokers as compared with

current smokers (Bickel et al., 1999) could result from reversible effects of nicotine as suggested by the authors. However, the lower levels of impulsivity in ex-smokers could also result from their more self-controlled predispositions that enabled them to quit smoking. For this reason, animal models are valuable to better understand effects of impulsivity on nicotine's actions. Research on the association between nicotine and impulsivity is summarized in Table 1.

Diergaarde et al. (2008) reported that impulsive action and impulsive choice predict different stages of nicotine-seeking in rats. Impulsive action was associated with an enhanced motivation to initiate and maintain nicotine self-administration (Diergaarde et al., 2008). Therefore, impulsive action was associated with the beginning stages of the addiction cycle (i.e., acquisition, binge/intoxication). Differences in nicotine reinforcement may contribute to the association between impulsivity and initiation of nicotine self-administration. Reinforcing actions of nicotine relevant to initiation and maintenance were examined in a rat model of impulsivity in the present research.

Results from Perkins et al. (2008) suggest that initial sensitivity to nicotine reward and reinforcement was associated with impulsive characteristics related to novelty seeking, response disinhibition, and extraversion. Factors associated with smoking initiation and maintenance were examined in the present research.

Table 1. Impulsive action and impulsive choice as they relate to phases of drug addiction.

| Drug Addiction Phase | Impulsive Action | Impulsive Choice |
|---|---|---|
| **Initiation** | Predicts acquisition of nicotine self-administration in rats (Diergaarde et al., 2008) | Predicts rate of acquisition of cocaine self-administration in rats (Perry et al., 2005, 2008) |
| | Predicts acquisition of cocaine self-administration in rats (Belin et al., 2008) | |
| | Related to initial sensitivity to nicotine reward in humans (Perkins et al., 2008) | |
| | Was higher in rats that later self-administered more cocaine than low-impulsivity rats (Dalley et al., 2007) | |
| **Maintenance** | Predicts maintenance of nicotine self-administration in rats (Diergaarde et al,, 2008) | Is higher in cigarette smokers than non-smokers (Mitchell, 1999) and ex-smokers (Bickel et al., 1999) |
| **Cessation** | | Is associated with nicotine reinstatement and nicotine-seeking during abstinence (Diergaarde et al., 2008) |
| | | Predicts reinstatement of cocaine-seeking in rats (Perry et al., 2008). |

The finding that impulsive action predicted an enhanced motivation to initiate and maintain nicotine self-administration (Diergaarde et al., 2008) is consistent with its neurobiological mechanism. Impulsive action is mediated by increased DA release (van Gaalen et al., 2006b). Increased DA neurotransmission is associated with increased drug-seeking and self-administration (Robinson & Berridge, 2008). This association is consistent with the report by Diergaarde et al. (2008) that impulsive action, which is mediated by increased DA neurotransmission, predicts acquisition and maintenance of

nicotine self-administration. It was hypothesized in the present research that nicotine reinforcement and impulsive action will be higher in Lewis rats than in Fischer rats, two rat strains that differ in impulsivity and DA neurotransmission. It was further hypothesized that stress, which increases DA neurotransmission, also will increase impulsive action and nicotine reinforcement in the two rat strains. Because Lewis rats have higher levels of impulsivity and DA neurotransmission, it was hypothesized that stress will increase impulsive action to a greater degree in Lewis than in Fischer rats.

## Impulsivity, Tobacco Use, and Incentive Sensitization

The relation between impulsivity and drug use or impulsivity and nicotine use has been established. However, with the exception of a few studies (Doran et al., 2006, 2007), psychological mechanisms underlying these associations have not been addressed experimentally. Doran and colleagues reported that nicotine provided greater relief from negative affect (Doran, McChargue, Spring, VanderVeen, Cook, & Richmond, 2006) and exposure to nicotine cues elicited greater cigarette craving (Doran, Spring, & McChargue, 2007) in impulsive smokers compared with non-impulsive smokers. Increased cigarette craving in response to smoking cue exposure in impulsives is consistent with greater incentive sensitization, a psychological mechanism of drug abuse, in impulsive individuals.

If incentive sensitization were augmented in impulsive individuals, then they would have increased nicotine reinforcement, which would increase their

liability to use and become addicted to nicotine. The present research determined whether nicotine behavioral sensitization, a rat model of incentive sensitization, is higher in Lewis (impulsive) rats than in Fischer (non-impulsive) rats. Further, the present research determined whether stress affects nicotine behavioral sensitization differentially in impulsive and non-impulsive rats.

*Incentive Sensitization Theory*

When an individual is addicted to a drug, cues or "incentives" associated with drug use become particularly salient to the addicted person, grabbing his or her attention and eliciting feelings of wanting and craving. Robinson and Berridge (1986, 1993, 2000, 2003) suggested that the increased salience of drug incentives results from effects of repeated drug use on the neurobiology of the addicted individual. According to the Incentive Sensitization Theory of Addiction (Robinson & Berridge, 1986, 1993, 2000, 2003), addiction results from progressive and persistent neuroadaptations that occur in response to repeated administration of a drug. Robinson and Berridge (1993) proposed that the brain system that mediates incentive motivation and reward is among those systems affected by repeated drug use. The system progressively becomes hypersensitized to drugs and to stimuli associated with drug use, so that they become more salient and attractive to the user. As sensitization of this neural system progresses, the ability of drug-associated stimuli to control behavior increases so that a compulsive pattern of drug use emerges (Robinson & Berridge, 1993).

Importantly, Robinson and Berridge distinguished between motivational and affective aspects of the drug experience. They identify "wanting" as the incentive motivational process and "liking" as pleasurable effects that occur from drug consumption (Berridge, 2001). The neural systems that mediate drug "wanting" (motivational process of incentive salience) and "liking" are dissociable, and incentive sensitization affects the neural system that mediates "wanting" (Berridge, 2001; Pecina et al, 2003; Robinson & Berridge, 2008).

Diverse research areas indicate that incentive sensitization occurs in humans, including attentional bias for drug-related stimuli (e.g., Boileau, Dagher, Leyton, Gunn, Baker, Diksic, & Benkelfat, 2006; Robinson & Berridge, 2008; Wiers & Stacy, 2006). Attentional bias for drug-related stimuli predicts relapse to cigarette smoking (Waters et al., 2003) and to alcohol use (Cox et al., 2002) in individuals attempting to quit. It has been suggested that the salience of incentives may be altered in impulsive individuals (Dawe, Gullo, & Loxton, 2004), which was examined in the present research using the behavioral sensitization phenomenon as a rat model of incentive sensitization.

*Behavioral Sensitization*

Sensitization to many psychostimulant drugs is manifested behaviorally in experimental animals, and provides a useful analog of the incentive sensitization phenomenon underlying addiction (Stewart & Badiani, 1993; Vanderschuren & Kalivas, 2000, Robinson & Berridge, 1993). In animal models, behavioral sensitization is a progressive and incremental increase in drug effects, including locomotion, that occurs in response to repeated administration of

psychostimulant drugs such as nicotine (e.g., Booze, Welch, Wood, Billings, Apple, & Mactutus, 1999; Clark & Kumar, 1983; DiFranza & Wellman, 2007; Harrod, Mactutus, Bennett, Hasselrot, Wu, Welch, & Booze, 2004; Robinson & Berridge, 1993; Vanderschuren & Kalivas, 2000). In particular, increases in horizontal activity indicate behavioral sensitization (DiFranza & Wellman, 2007, Robinson & Berridge, 1993). Increased locomotor activity, or ambulation, typically occurs in response to administration of a low-to-moderate psychostimulant dose, and increases gradually with repeated drug administration.

The neural basis of behavioral sensitization involves a hypersensitivity of mesotelencephalic DA systems (Robinson & Berridge, 1993). Several lines of evidence support changes in DA neurotransmission as the neurobiological mechanism of behavioral sensitization (Wise, 1987; Hamamura et al., 1991; Kalivas & Stewart, 1991). Increased DA neurotransmission in impulsives may make them more vulnerable to behavioral sensitization.

The increase in locomotor activity that occurs in behavioral sensitization reflects an increased motivation to consume the drug (Robinson & Berridge, 1993), with several lines of evidence supporting this interpretation and indicating that behavioral sensitization enhances drug reinforcement and motivation to consume a drug (e.g., Piazza, Deminiere, le Moal, & Simon, 1990; Lett, 1989). Further, several lines of direct evidence indicate that behavioral sensitization reflects incentive sensitization (Robinson & Berridge, 2008, Berridge, 2002;

Cardinal et al., 2002; Harmer & Phillips, 1998; Wyvell & Berridge, 2001; Taylor & Horger, 1999; Di Ciano, 2007).

Many factors affect susceptibility to psychostimulant sensitization, including sex, experience, and genetics. Female rats have greater behavioral sensitization than do male rats to many drugs, including cocaine (Carroll et al., 2007; Harrod et al., 2005), nicotine (Perna et al., 2008), amphetamine, and methamphetamine (Milesi-Hallea et al., 2007). Prior stress exposure increases behavioral sensitization to psychostimulants, a phenomenon termed "cross-sensitization" (Phillips, Roberts, & Lessov, 1997; Antelman, Eicher, Black, & Kocan, 1980). Environment also can affect behavioral sensitization to psychostimulants. Environmental enrichment has been reported to decrease behavioral sensitization in rodents to many drugs, including cocaine (Solinas, Chauvet, Thiriet, Rawas, & Jaber, 2008), amphetamine (Bardo, Bowling, Rowlett, Manderscheid, Buxton, & Dwoskin, 1995), and nicotine (Greene, Cain, Thompson, & Bardo, 2003). Genetics also can affect psychotimulant sensitization, with susceptibility to sensitization differing between genetic rat strains (Glick, Shapiro, Drew, Hinds, & Carlson, 1986; Leith & Kuczenski, 1982). Genetic rat strain differences in behavioral sensitization were explored in the present experiments using the Lewis and Fischer rat strains. Individual differences in sensitization are important because, according to Incentive-Sensitization theory, factors that affect susceptibility to sensitization also will contribute to individual differences in susceptibility to addiction (Robinson & Berridge, 2000).

*Incentive Sensitization and Impulsivity*

It is possible that sensitization of incentive salience is increased in impulsive individuals (Dawe, Gullo, & Loxton, 2004), which would contribute to the greater susceptibility to addiction associated with impulsivity. Because incentive sensitization affects the neural system that mediates wanting, greater incentive sensitization in impulsive individuals would imply that they have a greater wanting for drugs or other appetitive stimuli. Preclinical research supports this possiblity. Robinson and Berridge (1993) hypothesized that a sensitized incentive salience process leads to compulsive patterns of drug-seeking behavior. As discussed earlier, Belin et al. (2008) reported that impulsivity predicts the transition to compulsive cocaine-taking in rats. If incentive sensitization underlies the emergence of compulsive behavior (Robinson and Berridge, 1993) and impulsivity predicts the transition to compulsive drug-taking (Belin et al., 2008), then incentive sensitization should be increased in impulsives.

## Psychological Stress

Psychological stress is another factor that influences drug use. Psychological stress is experienced when an organism perceives that a real or imagined challenge or threat exceeds his or her resources for coping (Baum et al., 1981, 1982, 1997). Stress influences self-administration of a variety of drugs (Grunberg et al., 2010), including cocaine (Goeders, 2002), opiates (Shaham & Stewart, 1995; Shaham, et al., 2003), and alcohol (e.g., Hilakivi-Clarke & Lister,

1992). Most relevant to the present experiments, stress is associated with the initiation and maintenance of tobacco use (Kassel, et al., 2003).

*The Stress Response*

The experience of psychological stress sets in motion a cascade of physiological events involving the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) (McEwen, 2000). Activation of these systems mobilizes energy and prepares an organism to meet a challenge (fight) or to flee from the challenge (flight) (Cannon, 1929; Selye, 1936).

HPA axis activation begins with release of Corticotrophin Releasing Factor (CRF) from the hypothalamus. When CRF is released from the hypothalamus, it is detected by the anterior pituitary, which then releases adrenocorticotrophin hormone (ACTH) into the blood stream. This hormone is detected by the adrenal glands, which release cortisol into the bloodstream. Cortisol mobilizes energy in the form of glucose by breaking down adipose tissue to prepare the body for a fight-or-flight response. Corticosterone, the equivalent of cortisol in rodents, is often measured in experiments in which stress is manipulated to verify that stress occurred. In the present research, corticosterone levels were measured to examine rat strain differences in stress responses and as a manipulation check to provide verification of stress induction.

The sympathetic nervous system (SNS) is a branch of the autonomic nervous system that is activated in response to stress, mediating the fight-or flight response (Cannon, 1929). Activation of the SNS stimulates release of the catecholamines epinephrine (adrenaline) and norepinephrine from the adrenal

medulla into the bloodstream. Activation of the SNS and release of the catecholamines preserves homeostasis by preparing the organism for anticipated exertion. Effects of SNS activation include increased cardiovascular and respiratory function and increased energy mobilization. During SNS activation, non-emergency functions mediated by the parasympathetic nervous system, such as digestion and reproduction, are suppressed.

Psychological stress increases dopamine release in rats in brain regions implicated in drug abuse, including the striatum, nucleus accumbens, and medial prefrontal cortex (e.g., Abercrombie, Keefe, DiFrischia, & Zigmond, 1989; Finlay, Zigmond, & Abercrombie, 1995). Increased dopamine neurotransmission increases impulsive action (van Gaalen et al., 2006). Therefore, it was hypothesized that stress would increase impulsive action in the present research.

Psychological stress also increases DA release in humans, although results in human studies are mixed. Pruessner, Champagne, Meaney, and Dagher (2004) reported significant psychosocial stress-induced release of DA in the ventral striatum of human participants who had received low early-life maternal care, although Montgomery, Lingford-Hughes, Egerton, Nutt, and Grasby (2006) reported no significant increase in DA release in the striatum of healthy human participants in response to a mild psychological stressor.

However, the effect of the glucocorticoid cortisol on DA release in humans becomes clearer in the presence of a psychostimulant challenge. Oswald et al. (2005) reported that higher cortisol levels augmented DA release in response to amphetamine administration in healthy male and female adults. Subjects with

higher cortisol levels and greater DA release had higher ratings of positive response to amphetamine administration (Oswald et al., 2005). This research suggests that stressed individuals may be more reinforced by drug use. An effect of stress to increase the reinforcement value of drugs is a potential psychological mechanism that was examined in the present research.

### *Stress and Drug Use*

Not only does stress exposure increase vulnerability to drug addiction, but drug escalation *per se* activates the HPA axis. Research indicates a role of stress hormones in all aspects of drug self-administration, including acquisition of self-administration, maintenance of self-administration, and reinstatement (Piazza & LeMoal, 1998; Goeders, 2002; Koob & Kreek, 2007). The role of CRF in cocaine escalation was revealed by Specio et al. (2008), who attenuated escalated cocaine self-administration by administering a CRF-1 receptor antagonist. Additionally, CRF affected motivated behavior in an ethanol self-administration paradigm, with CRF-1 receptor antagonists dose-dependently decreasing ethanol self-administration during withdrawal in dependent rats (Valdez et al., 2002). Because stress hormone activation plays a critical role in all aspects of drug addiction, it follows that psychological stress would augment vulnerability to drug addiction. The present research examines effects of stress on reinforcing actions of nicotine because it is possible that such effects may contribute to the role of stress in drug addiction. Further, this effect may be augmented in impulsive individuals, making them more vulnerable to the stress and drug use relationship.

Research in humans and animals reveal effects of psychological stress on addiction. In humans, stress is associated with increased cigarette smoking (George et al., 2007; Grunberg & Baum, 1985; Jarvik et al. 1977; Kassel et al., 2003). Individuals exposed to stress are more likely to abuse drugs or undergo drug relapse (Brewer et al., 1998; Sinha et al., 2000). In animals, increases in corticosterone (the rat equivalent of cortisol) or in sensitivity to corticosterone increases vulnerability to addictive effects of drugs of abuse (Piazza & LeMoal, 1998). The effects of stress on drug abuse in animal models are evident in each phase of the addiction cycle. In rodents, stress increases drug-seeking, drug-craving, and self-administration of substances, including nicotine (Buczek et al., 1999; Grunberg et al., 2010; Le et al., 1998; Shaham et al., 1993; Soloff, Lynch, & Moss, 2000). Effects of stress on reinforcing actions of nicotine, which are examined in the present study, may underlie these relationships.

*Psychological theories of stress and drug use*

In addition to physiological and neurobiological mechanisms, stress also may impact drug addiction through associated psychological mechanisms. According to Incentive Sensitization theory, increased drug motivation results from persistent neuroadaptations from repeated drug use in the neural systems that underlie incentive salience and reinforcement (Robinson & Berridge, 1993). In a phenomenon called cross-sensitization, prior sensitization to drugs causes hyper-responsiveness to stress, and prior sensitization to stress causes hyper-responsiveness to drugs (e.g., Antelman & Chiodo, 1983; Antelman, Eichler, Black, & Kocan, 1980). If prior experience with stress or drugs caused hyper-

responsiveness to drugs or stress, respectively, then it is likely that acute stress will cause hyper-responsiveness to drugs as well. Therefore, it was hypothesized in the present research that stress would increase nicotine behavioral sensitization.

Effects of stress and drug cross-sensitization are also evident in the incentive motivational properties of drugs (Robinson & Berridge, 1993; Goeders, 2002). Prior stress facilitates the acquisition of amphetamine (Piazza, et al., 1990) and cocaine self-administration (Goeders & Guerin, 1994) in rats. Rouge-Pont, Marinelli, Le Moal, Simon, and Piazza (1995) demonstrated in a rat model that effects of stress on cocaine sensitization depend on corticosterone secretion. A corticosterone inhibitor suppressed cocaine-induced sensitization of accumbens DA release. Additionally, the corticosterone inhibitor suppressed the effects of cocaine to increase locomotor activity, which was measured once following cocaine administration. It is possible that stress may increase reinforcing actions of nicotine, as measured by the behavioral sensitization paradigm. Further, effects of stress on reinforcing actions of nicotine may be increased in impulsive individuals compared to non-impulsive individuals. Such effects could underlie the effects of stress on drug use, and the association between impulsivity and drug use.

### *Summary of the Literature*

Impulsivity is an important construct to understand because of its role in detrimental human behaviors. While impulsivity is considered a stable trait or

temperament, levels of impulsivity can vary from moment to moment. Stress may increase state impulsivity, and this effect may be greater in individuals with higher levels of trait impulsivity. This possibility is examined in the present research.

Cigarette smokers are more impulsive than non-smokers (e.g., Mitchell, 1999). Diergaarde et al. (2008) reported that impulsivity statistically predicted nicotine self-administration in rats (e.g., Diergaarde et al., 2008), but the psychological mechanism underlying this relation is unknown. It is possible that incentive sensitization is increased in impulsive individuals, as was examined in the present research.

In addition to impulsivity, psychological stress is a factor that augments nicotine use. Stress hormones play a role in many aspects of drug use (e.g., Koob & Kreek, 2007). Additionally, psychological stress increases self-administration, seeking, and craving of drugs, including nicotine, in humans and in animal models. Effects of stress to increase nicotine incentive sensitization may underlie this relationship. This possibility was examined in the present research using an animal model of incentive sensitization, the behavioral sensitization paradigm.

### *Research Questions*

It is known that: (1) impulsive action predicts initiation and maintenance of nicotine self-administration in rats (Diergaarde et al., 2008), and (2) acute stress exposure enhances self-administration of the psychostimulants cocaine (e.g.,

Goeders & Guerin 1994) and amphetamine (e.g., Piazza et al. 1990) and reinstates nicotine-seeking in rats (Buzcek et al., 1999). Whether increased nicotine reinforcement is the mechanism by which impulsive action is related to nicotine self-administration is not known. Further it is not known whether: (1) stress increases impulsive action, (2) stress affects behavioral sensitization to nicotine differentially in impulsive and non-impulsive rats, or (3) repeated nicotine administration affects impulsivity differentially in impulsive and non-impulsive rats. These research questions are relevant to understand why impulsivity is associated with increased cigarette smoking, and why cigarette smoking increases under stress. The present research was conducted to determine effects of repeated acute stress on impulsive action and nicotine behavioral sensitization in impulsive and non-impulsive rats. Addressing these research questions provides information relevant to smoking treatment and prevention strategies in stressed and impulsive humans.

*Conceptual Model: Impulsivity, Stress, and Behavioral Sensitization to Nicotine*

Established and predicted relations among impulsivity, stress, and behavioral sensitization to nicotine are depicted in the conceptual model below (Figure 1 on page 35). In the model, variables examined in the present research are emphasized with bolded text and boxes (i.e., stress, impulsive action, behavioral sensitization to nicotine).

The hypothesized effect of repeated acute stress to increase impulsive action and behavioral sensitization to nicotine is depicted in the model.

Predictions about effects of stress on impulsive action and nicotine behavioral sensitization are based on reported effects of stress increasing dopamine neurotransmission (Abercrombie et al., 1989; Finlay et al., 1995; Oswald et al., 2005; Pruessner et al., 2004). Increased dopamine neurotransmission is the neurobiological mechanism underlying impulsive action (van Gaalen et al., 2006a) and behavioral sensitization to psychostimulants (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987). Because stress increases dopamine neurotransmission, it was hypothesized that stress will increase impulsive action and behavioral sensitization to nicotine. The predicted effects are depicted in the conceptual model in Figure 1. In the present research, the same rats used in Experiment 1 were used in Experiment 2, with the stressed rats in Experiment 1 also receiving stress in Experiment 2. Because the rats stressed in Experiment 2 had been previously stressed in Experiment 1, it is not possible to separate effects of the acute, recurrent stress of Experiment 2 from the stress that occurred previously in the rats' history.

The hypothesis that impulsive action and behavioral sensitization to nicotine will be greater in the Lewis rat strain is depicted in the conceptual model. Predictions about effects of rat strain on impulsive action are based on reports of differences between Lewis and Fischer rats on impulsive choice (Andersen & Woolverton, 2005) and measures related to impulsive action (Kearns et al., 2006). Predictions about effects of rat strain on nicotine behavioral sensitization are based on rat strain differences in behavioral sensitization to nicotine observed in the previously conducted experiment, reported below. These

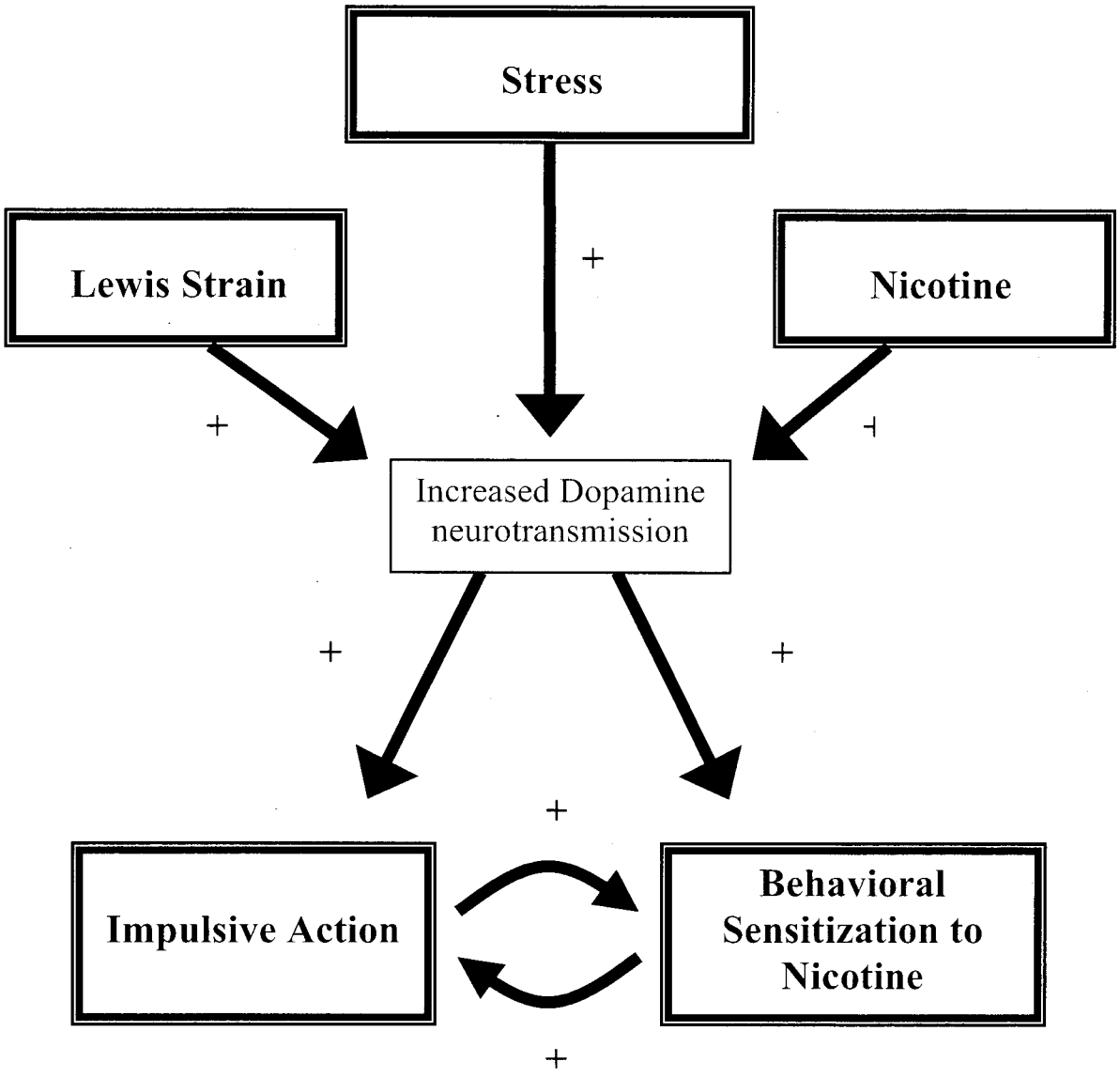
predictions also are based on reports of increased dopamine neurotransmission in Lewis rats, which are discussed below (Camp et al., 1994; Flores, et al., 1998; Kosten & Ambrosio, 2002; Strecker et al., 1995).

Nicotine is depicted in the model because it was administered to all rats in the research. Effects of nicotine administration to increase dopamine release and cause behavioral sensitization are depicted in the model. These predicted effects are based on research reporting nicotine's effects to increase DA release (e.g., Imperato et al., 1986) and cause behavioral sensitization (e.g., DiFranza & Wellman, 2007; Booze, et al., 1999; Clark & Kumar, 1983; Harrod, et al., 2004).

It was predicted that effects of repeated acute stress on impulsive action and nicotine behavioral sensitization will be correlated with each other. This positive correlation is represented in the conceptual model by two curved arrows with positive valences between impulsive action and nicotine behavioral sensitization.

It was predicted that relations among stress, impulsive action, and nicotine behavioral sensitization will have a greater effect in trait impulsive individuals. For this reason, it was predicted that effects of stress on impulsive action and nicotine behavioral sensitization will be greater in impulsives.

Figure 1. Conceptual Model



## *Preliminary Work: The Effect of Stress on Nicotine Behavioral Sensitization in Lewis and Fischer Rats*

A preliminary experiment was conducted to begin to examine effects of stress on behavioral sensitization to nicotine in male Lewis and Fischer rats, the rat strains that were used in the present experiment. This preliminary experiment did not measure impulsive action. Psychological stress increased behavioral sensitization to repeated daily injections of nicotine during the first three days of nicotine administration in Lewis and Fischer rats, and the effect was especially pronounced in Lewis rats. This preliminary work was conducted to develop the nicotine behavioral sensitization technique and to determine the logistics and feasibility of inducing sensitization in Lewis and Fischer rats in preparation for the larger research project, in which impulsive action was measured.

*Purpose.* The aims of this preliminary experiment were to determine the effects of trait impulsivity and psychological stress on behavioral sensitization to the stimulant nicotine in Lewis and Fischer rats. Two genetic rat strains that differ on measures of impulsive choice and impulsive action were used: the "impulsive" Lewis rats, and the "non-impulsive" Fischer rats.

*Hypothesis.* It was hypothesized that stress would increase behavioral sensitization to nicotine and that this effect would be greater in the Lewis rat strain.

*Subjects.* 64 adult male rats (32 Fischer, 32 Lewis), aged 40 days at the start of the experiment.

*Design*. The experiment was a 2(stress) x 2(rat strain) x 2(drug) factorial design with repeated measures. All rats initially received daily injections of 1 ml of saline for 3 days to acclimate them to the injection procedure. Rats then received 0.5 mg/kg doses of nicotine or saline via subcutaneous (SC) injection daily for 14 days. Behavioral sensitization to nicotine was measured in open field chambers immediately after injections.

*Data analysis.* Horizontal activity, total distance traveled, and stereotypy data from the first seven days of drug administration were analyzed with repeated-measures Analysis of Covariance (ANCOVA), with baseline behaviors as the covariate. The first seven days of drug administration were analyzed because the behavioral sensitization to nicotine is maximal during the first 5-7 days of administration (Kempsill & Pratt, 2000; DiFranza & Wellman, 2007). Results are presented in Figures 2a and 2b below.

*Results*. Time x drug interactions on the horizontal activity [$F(3, 117)=20.17$, $p<0.001$] parameter revealed that behavioral sensitization occurred to nicotine. Time x rat strain x drug interactions on the horizontal activity [$F(3, 117)=2.86$, $p<0.05$] parameter indicated that behavioral sensitization to nicotine was greater in Lewis rats than in Fischer rats. As seen in Figures 2a and 2b, a main effect of stress occurred during the first seven drug days only in the Lewis rats [$F(1,19)=6.492$ $p<0.025$], indicating that Lewis and Fischer rats respond differently to stress, regardless of drug condition. Drug Day Three seemed to be an important day in terms of effects of stress and rat strain. There was a large amount of variance on Drug Day 3 in the stressed Lewis and Fischer groups that

received nicotine, although Mauchly's Test of Sphericity was not violated in the repeated-measures ANCOVA.

*Conclusions*. Hypotheses were supported by these results. Stress increased behavioral sensitization to nicotine in Lewis rats on the third day of drug administration. While it is possible that impulsivity is the variable that influenced behavioral sensitization to nicotine, this conclusion could not be made unequivocally because impulsivity was not measured. Because the large amount of variance observed in stressed rats that received nicotine in the present experiment could increase the chance of a Type 1 error, these results required replication.

The present research was designed to build upon this preliminary experiment. In the larger research project, impulsive action was measured using the five-choice serial reaction time task (5-CSRTT). This behavioral measurement of impulsivity allows for the determination of whether impulsivity predicts behavioral sensitization to nicotine. Specifically, the impulsive action of each individual rat is quantified into an impulsivity index, and that index is used to determine whether impulsive action statistically predicts response to nicotine. The present research was designed to further examine this question, as well as to examine effects of stress on impulsive action in a rat model of impulsivity. In addition, the present research replicated rat strain differences in the effects of stress on behavioral sensitization to nicotine.

Fig. 2a. Preliminary Experiment Lewis Horizontal Activity

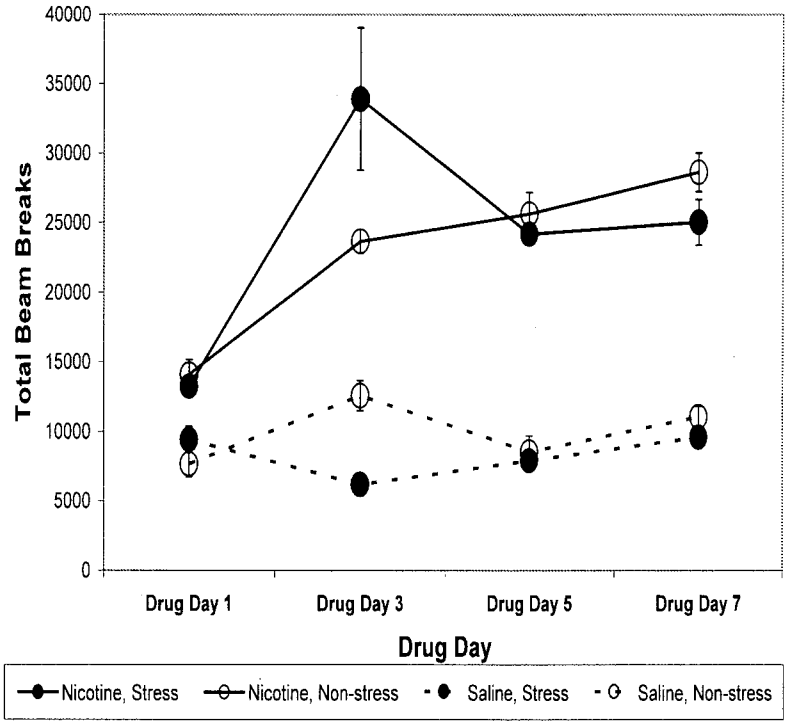
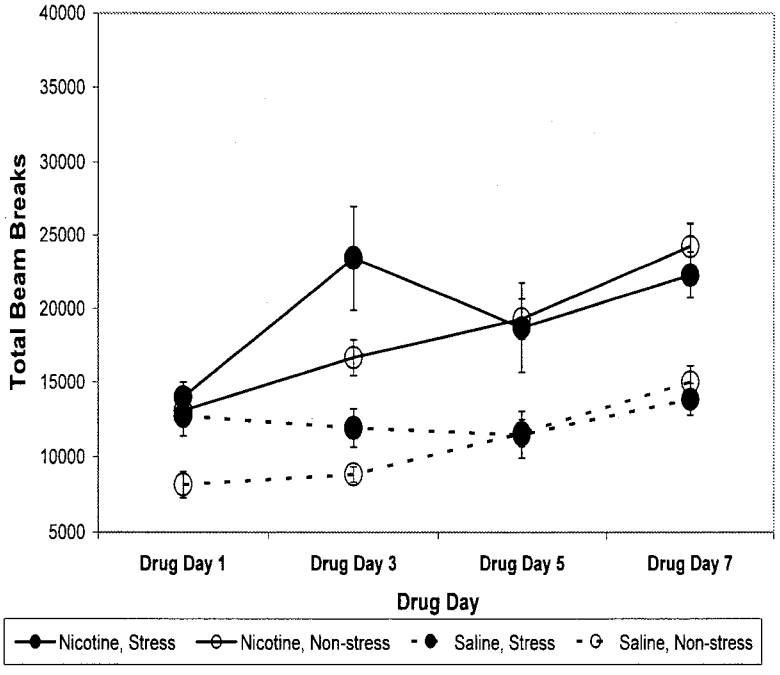



Fig. 2b. Preliminary Experiment Fischer Horizontal Activity



## DOCTORAL RESEARCH PROJECT

The present research was designed to evaluate the effect of acute stress on state impulsivity, and to examine the effect of stress on sensitization to nicotine in a rodent model of impulsivity. In Experiment 1, effects of stress on impulsive action were measured in a rat model of impulsivity using the Five Choice Serial Reaction Time Task (5-CSRTT). In Experiment 2a, the effects of stress and impulsive action on behavioral sensitization to nicotine were examined in a rat model of impulsivity. In Experiment 2b, effects of stress and behavioral sensitization to nicotine on impulsive action and attention were measured in the same rats that were used in Experiment 2a. The behavioral measure of impulsivity allows for statistical assessment of the influence of impulsivity on each dependent variable measured. The same rats were used in both experiments so that the initial level of impulsivity measured in each rat in Experiment 2a could be used to predict their nicotine-induced locomotor activity in Experiment 2b. The experiments fit together to examine effects of stress on impulsive action and reinforcing actions of nicotine, and the ways these effects may differ among impulsive and non-impulsive individuals. Fischer and Lewis rats were used as an animal model of impulsivity, with Lewis rats as the more impulsive rat strain.

### *Rat model of impulsivity: Lewis and Fischer Rats*

Animal models are valuable because they allow for the examination of drug effects in a true experiment. Lewis and Fischer (F-344) rats provide a rat model of impulsivity. Anderson and Woolverton (2005) reported that Lewis

and F-344 rats differ in delay discounting, an operant choice task often used as a measure of impulsivity. Lewis rats display higher rates of delay discounting than F-344 rats, indicating that Lewis rats are more impulsive than F-344 rats on measures of impulsive decision making. Additionally, Kearns et al. (2006) found that Lewis rats demonstrate more autoshaping, which is considered to be related to impulsive action.

The 5-CSRTT provides a reliable measure of impulsive action, with premature responses indexing impaired response inhibition (Winstanley et al., 2004a; 2006). Impulsive action is a focus of the present research because it is relevant to initiation of cigarette smoking. In the 5-CSRTT, rats are required to inhibit a prepotent response until the presentation of a visual target. Use of the 5-CSRTT in the present experiments provided further validation of Lewis and Fischer rats as an animal model of impulsivity, and determined whether Lewis rats perform more impulsively on a measure of impulsive action.

Just as nicotine use is correlated with impulsivity in humans (e.g., Mitchell, 1999), Lewis and F-344 rats show differences in nicotine intake and preference. Lewis rats self-administer more nicotine than Fischer rats (Brower, Fu, Matta, & Sharp, 2002). Lewis rats are more sensitive to nicotine. They discriminate lower doses of nicotine in a discrimination task than Fischer rats (Philibin et al., 2005). Nicotine is more appetitive and less aversive to Lewis rats than Fischer rats. Lewis rats developed conditioned place preference (CPP) to a location in which nicotine was administered repeatedly while Fischer rats did not (Horan, Smith, Gardner, Lepore, & Ashby, 1997; Philibin et al., 2005), which may indicate

increased incentive sensitization to nicotine in Lewis rats. In fact, when Horan et al. (1997) increased the number of nicotine injections in one location to ten pairings, Fischer rats developed conditioned place *aversion.* When Lewis and Fischer rats were injected with nicotine during saccharine consumption, Fischer rats acquired taste aversion faster and to a greater degree than did Lewis rats (Pescatore, Glowa, & Riley, 2005), indicating that they like nicotine less than do Lewis rats. Further, nicotine withdrawal is more aversive for Lewis rats. Lewis rats chronically-infused with nicotine developed conditioned place aversion after receiving injections of the nicotine receptor antagonist, mecamylamine, but Fischer rats treated identically did not (Suzuki, Ise, Maeda, & Misawa, 1999). Although differences between Lewis and Fischer rats in nicotine intake and preference are robust, differences in nicotine behavioral sensitization have not been examined in Lewis and Fischer rats. It was hypothesized in the present experiment that behavioral sensitization to nicotine will be greater in the Lewis rats than the Fischer rats. If confirmed, then this information will support the interpretation that incentive sensitization to nicotine underlies differences in nicotine preference in Lewis and Fischer rats.

There are mesolimbic DA differences between Lewis and Fischer rats (Kosten & Ambrosio, 2002) that correspond to mesolimbic DA differences in impulsive and non-impulsive humans (e.g., Eisenberg et al., 2007). Among the differences, Lewis rats show a more prolonged elevation in DA levels following methamphetamine and cocaine administration (Camp et al., 1994; Strecker et al., 1995). Lewis rats have lower nucleus accumbens DA D2 and D3 receptor

densities than do Fischer rats (Flores et al., 1998). Additionally, Lewis rats have lower levels of dopamine transporters (DAT) in the nucleus accumbens compared to Fischers (Flores et al., 1998). DA transporters are responsible for clearing DA from the synapse and terminating the DA signal, so lower levels of DAT lead to prolonged elevation of DA levels. Each of the described differences in DA neurotransmission is likely to predispose Lewis rats to impulsivity, which is consistent with reported behavioral differences (Anderson & Woolverton, 2005; Kearns et al., 2006).

In addition to differences in nicotine intake, preference, and sensitivity, Lewis and Fischer rats also differ with regard to hypothalamic-pituitary-adrenal (HPA) axis response to stress. Lewis rats are hyporesponsive to stress as compared with Fischer rats, as reflected by lower corticosterone and adrenocorticotrophin (ACTH) levels both in response to a stressor (Chaouloff et al., 1995; Dhabar et al., 1993) and at rest (Dhabar et al., 1993). Fischer rats have augmented biochemical responses to stress compared with Lewis rats. Rat strain differences in stress responsivity should be considered when comparing effects of stress on impulsive action in Lewis and Fischer rats.

It is noteworthy that Lewis rats are more impulsive than Fischer rats, but are less sensitive to stress. While little research has compared stress reactivity in impulsive and non-impulsive individuals, research examining stress reactivity in individuals with Attention Deficit Hyperactivity Disorder (ADHD), a disorder with an impulsivity component, has been conducted. Children with ADHD had lower levels of epinephrine in response to a challenge (Hanna, Ornitz, & Hariharan,

1996) as well as reduced salivary cortisol levels (Kariyawasam, Zaw, & Handley, 2002). Blunted stress responses may be a marker for a more developmentally pervasive form of ADHD (King, Barkley, & Barret, 1998). Further, lower HPA axis responsivity is related to impulsivity in ADHD. Hong and colleagues (2003) reported an association between blunted HPA axis reactivity and impulsivity in boys with ADHD. However, other research has reported increased stress reactivity in ADHD adults (e.g. Hirvikoski, Lindholm, Nordenstrom, Nordstrom, & Lajic, 2009; Lackschewitz, Huther, & Kroner-Herwig, 2008) with higher cortisol levels after a stressor related to higher impulsivity (Hirvikoski et al., 2009). The relationship between stress reactivity and impulsivity is unclear, although it seems that some type of stress dysregulation is associated with impulsivity. Whether impulsivity is related to increased or decreased stress reactivity should be examined in subjects without ADHD to rule out stress caused by impairments associated with ADHD.

It is likely that impulsivity and stress reactivity represent two orthogonal dimensions. This conceptualization is consistent with Gray's Reinforcement Sensitivity Theory (RST), in which anxiety and impulsivity comprise two separate emotional systems that motivate behavior (Gray, 1970). Because reports of associations between stress reactivity and impulsivity are mixed, differential stress reactivity in Lewis and Fischer rats do not detract from their validity as an animal model of impulsivity. In fact, differential stress reactivity in Lewis and Fischer rats may enhance, rather than undermine, the validity of the two rat strains as a model of impulsivity.

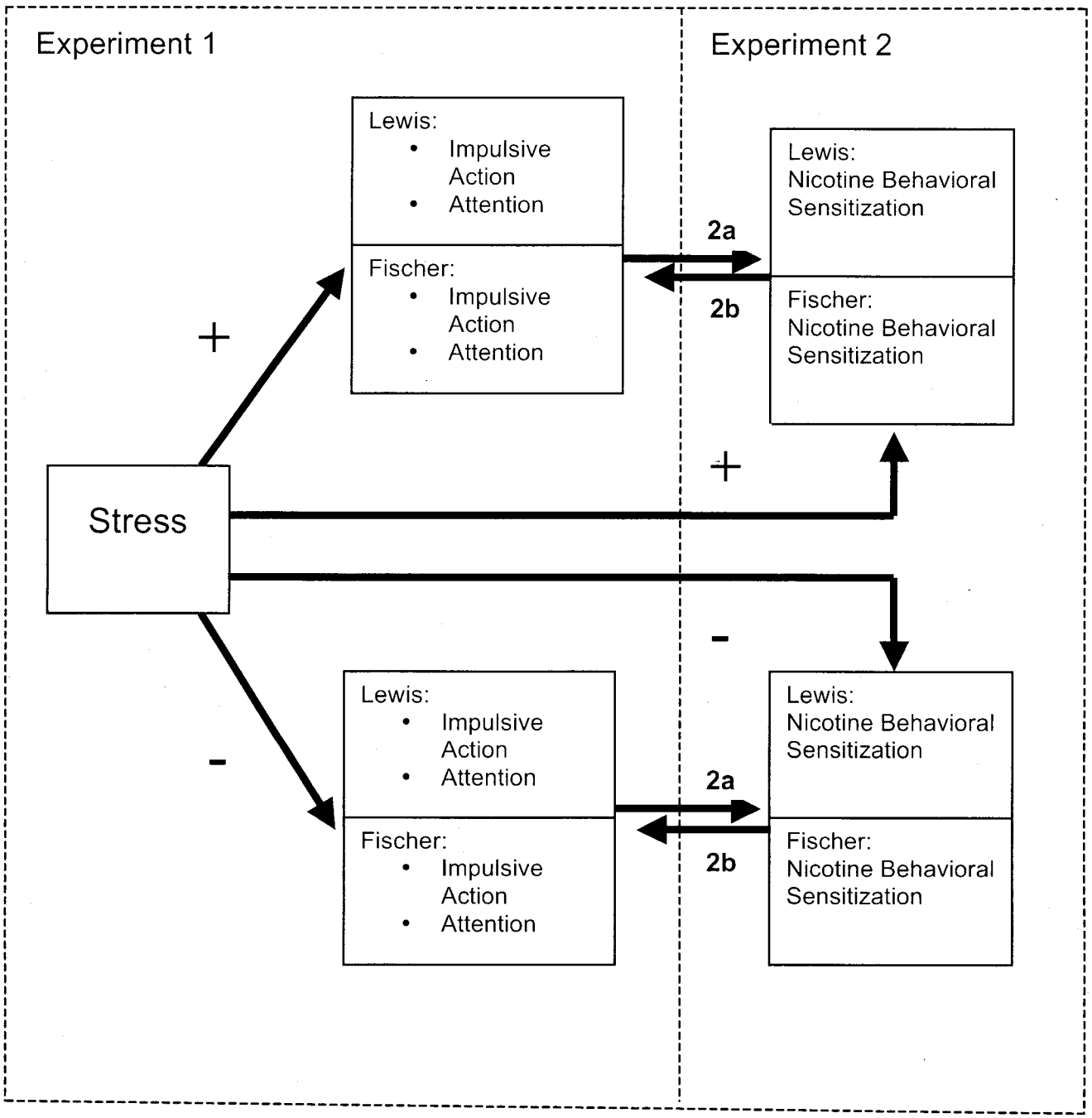
## Overview of present research

Cigarette smoking is increased in impulsive individuals, and is increased by psychological stress. Effects of stress on impulsivity and reinforcing actions of nicotine may mediate these relations. Psychological stress may increase impulsive action and reinforcing actions of nicotine, and these effects may be greater in individuals with a higher initial level of impulsivity. To examine these possibilities, psychological stress was manipulated in an animal model using two rat strains that differ in impulsivity, and the effects of stress on impulsive action, attention, and nicotine behavioral sensitization were determined in two related but conceptually distinct experiments (Figure 3). The two experiments were essentially two phases of one experiment because the same subjects were used, and were assigned to the same stress condition, in both experiments. However, the experiments are identified as Experiments 1 and 2 for clarity of communication.

In Experiment 1, the effect of stress on impulsive action and attention was examined in a rodent model of impulsivity using the operant 5-Choice Serial Reaction Time task (5-CSRTT). In Experiment 2, the effect of stress on behavioral sensitization to nicotine was determined in a rodent model of impulsivity by measuring locomotor activity daily after nicotine administration. In addition, the effect of nicotine sensitization on impulsive action and attention was examined in the (5-CSRTT). Links among stress, impulsivity, and reinforcing actions of nicotine were determined statistically. All experiments were run in

cohorts, with each experimental condition being equally represented. A timeline of Experiments 1 and 2 is presented in Appendix A.

Figure 3. Experimental Model. In this model, Experiment 1, Experiment 2a and Experiment 2b are represented.



# EXPERIMENT 1: The Effect of Stress on State Impulsivity in Lewis and Fischer Rats

## Methods

### *Overview*

Experiment 1 was designed to determine the effect of acute stress on impulsive action in Lewis and Fischer rats, two rat strains that differ on impulsivity with Lewis rats performing more impulsively on measures of impulsive action (Anderson & Woolverton, 2005). In Experiment 1, the effect of stress on impulsive action was tested on 32 rats in the Five Choice Serial Reaction Time Task (5-CSRTT). The experimental timeline is depicted in Appendix A.

### *Purpose*

Experiment 1 determined, in a rodent model of impulsivity, the effect of acute psychological stress on impulsive action as measured by the 5-CSRTT. Experiment 1 addressed Specific Aim 1.

### *Hypotheses*

It was hypothesized that impulsive action as measured by the 5-CSRTT will be greater in Lewis rats than in Fischer rats, that acute stress will increase state impulsive action in all rats, and that effects of stress and rat strain will combine so that impulsive action will be greatest in stressed Lewis rats.

**Hypothesis 1A**: Lewis rats will perform more impulsively than Fischer rats on the 5-CSRTT.

Rationale: Kearns et al. (2006) reported that Lewis rats demonstrate more autoshaping (sign-tracking) than Fischer rats, which is related to impulsive

action. For this reason, it was hypothesized that Lewis rats will have more premature responses on the 5-CSRTT, indicating that they have greater impulsive action.

**Hypothesis 1B**: Psychological stress will increase impulsive action in Lewis and Fischer rats.

Rationale: Psychological stress causes increased DA release, (Abercrombie et al., 1989; Finlay et al., 1995; Oswald et al., 2005; Pruessner et al., 2004). Increased dopamine (DA) neurotransmission causes increased impulsive action (van Gaalen, et al., 2006a). Therefore, it is hypothesized that stress will increase impulsive action in Lewis and Fischer rats.

**Hypothesis 1C**: The effect of stress to increase impulsive action will be greatest in Lewis rats.

Rationale: Lewis rats are more impulsive than Fischer rats (Kearns et al., 2006; Anderson & Woolverton, 2005). Lewis rats have increased DA neurotransmission compared to Fischer rats (Camp et al., 1994; Kosten & Ambrosi, 2002; Flores, et al., 1998; Strecker et al., 1995), which is a mechanism of impulsive action (van Gaalen et al., 2006). Stress causes increased DA release (Abercrombie et al., 1989; Finlay et al., 1995; Oswald et al., 2005; Pruessner et al., 2004). For these reasons, it was hypothesized that the effect of stress to increase impulsive action will be greatest in Lewis rats.

**Hypothesis 1D**: Lewis rats will have decreased attention compared with Fischer rats on the 5-CSRTT.

Rationale: Lewis and Fischer rats have not been compared on measures of attention in the 5-CSRTT or any other measure of attention in previous research. Impulsivity and attentional deficits are both components of Attention-Deficit Hyperactivity Disorder (ADHD) (DSM-IV-TR, 2000). It is possible that impulsivity and attentional deficits represent related constructs (Barkley, 1997). Because Lewis rats are more impulsive than Fischer rats on measures of impulsive choice and measures related to impulsive action, it is possible that Lewis rats also have decreased attention compared with Fischer rats. For this reason, it is hypothesized that Lewis rats will have decreased attention compared with Fischer rats. If confirmed, then Lewis rats may provide a rat model of ADHD.

### *Experimental Design*

Experiment 1 was a 2 (rat strain) x 2 (stress condition) full factorial design with repeated measures to allow the examination of between-subjects and within-subject variables, as well interactions among the variables. There was a total of N = 32 rats, with 8 rats per treatment cell.

### *Power analysis*

Sample size was based on power analyses performed using Java Applets for power and sample size (Lenth, 2006), and effect sizes from a study by Kearns et al. (2006) in which measures related to impulsive action (i.e., sign-tracking, discrimination reversal learning, and negative automaintenance) were examined in Lewis and Fischer rats. The sample size needed to detect the effect size

observed by Kearns et al. (2006) was then verified using a table of sample sizes required to detect hypothesized effect sizes in a 2 x 2 factorial between-subjects experimental design (Bausell & Li, 2002).

Calculations using data from the Kearns group indicated that the observed effect size (Cohen's d) of the main effect of rat strain was 1.25. A cell size of 6 rats (totaling 12 per rat strain) would allow for the detection of a main effect of rat strain with an effect size of 1.25 to be observed at 80% power with a significance level of $p < 0.05$. Effects of stress on impulsive action have not been examined. However, effects of stress on behavioral sensitization to nicotine were determined (Hamilton, Starosciak, & Grunberg, under review). In that study, the effect size of the main effect of stress was 1.45. A cell size of 5 would allow for the detection of a main effect of 1.45. However, the effect size of the stress by rat strain interaction in the previously conducted study was 0.46. A cell size of 38 rats would be needed to detect a stress x rat strain interaction with an effect size of 0.46. Because 38 rats per cell is not logistically feasible for the present research because of the amount of 5-CSRTT training required and costs if N=142 (N= 4 x 38), sample size calculations were based on that which is needed to detect main effects of stress and rat strain.

A cell size of 6 rats would be sufficient to determine main effects of stress and rat strain. However, there was a possibility of attrition occurring over the 10 to 12 weeks required for 5-CSRTT training. Additionally, in a study by Talpos et al. (2006) of impulsive action using the 5-CSRTT with Lister hooded rats, 90% of the rats trained met the training criterion (Robbins, personal communication,

2009). In a study examining Lewis and Fischer rats on measures related to impulsive action (Kearns et al., 2006), one Fischer rat did not meet the training criterion. To account for a decrease in power that could result from attrition or failure to meet a training criterion, a cell size of 8 rats was used in the present research.

*Oversampling of Fischer rats to ensure appropriate sample size.* It has been reported that Fischer rats do not always meet training criteria when performing operant tasks (Kearns et al., 2006). For this reason Fischers were oversampled, with four additional Fischer rats undergoing training on the 5-CSRTT; the additional rats were identified as A, B, C, and D. During training, one of the additional Fischers (B) died. While most of the Fischers learned the task, some of them performed poorly and did not meet all criteria. At the conclusion of training, the performances of all Fischer rats (including the three additional rats) were systematically evaluated to determine which would be included in the experiment. For all days in the final training phase of 1 second stimulus duration, total amount of responses on four parameters were recorded in a chart: total correct, total incorrect, omissions, and premature responses. Outliers reflected inadequate learning and performance on the task. Amount of total responses on any parameter that did not fall within the normal range of total responses was considered an outlier. For an individual rat, a total amount of correct responses that was less than 40 was considered an outlier. A total amount of incorrect responses, omissions, or premature responses for an individual rat that was greater than 20 also was considered to be an outlier. After

totaling occurrences of outliers for each rat within each parameter, the three rats with the greatest number of outlier occurrences during the final phase of training were excluded from the experiment. The excluded rats were 401, with 17 outlier occurrences in the final phase of training, and 201 and 202, each with 12 outlier occurrences during the last training phase. Because rats 201 and 202 were pair-housed together, they were replaced by the cage mates C and D. Rat A, which was housed singly after its cage-mate died, replaced 401 on all measures. However, the original rat 401 continued to be housed with 402 so as not to disrupt the pair-housing.

*Subjects and Housing*

At the beginning of the experiment, subjects were 16 adult male Lewis rats and 16 adult male Fischer rats (Charles River Laboratories), with a cell size of 8 rats. Upon arrival, all rats were approximately 26 days old, a young age, to allow time for rats to achieve the appropriate bodyweight and train on the 5-CSRTT before the experiment began. At the start of Experiment 1 (after 5-CSRTT training had concluded), all rats were approximately 144 days old; the Fischer rats' mean weight was 272.93 grams while the Lewis rats' mean weight was 385.13 grams. Male rats were used in the experiment because male rats are more impulsive than female rats (Jentsch & Taylor, 2003). Within rat strain, animals were pair-housed in standard rat cages (42.5 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri) with access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Crowded housing conditions (four male rats in a 32 x 20 x 18 cm cage) is stressful for male rats (Brown & Grunberg, 1995) but

individual housing also may be stressful for rats (e.g., Parker & Radlow, 1974). For this reason, rats were pair-housed within rat strain to avoid potentially stressful effects of crowding or isolation.

Animals were maintained at 85% to 90% of free-feeding body weight to motivate performance in the 5-CSRTT and to maintain health. Free-feeding body weight was determined by feeding *ad libitum* to an additional pair of Lewis rats and to an additional pair of Fischer rats (four rats total) of the same age as the experimental rats, and weighing them daily. The two additional pairs of rats were not part of the experiment, and were never used in any behavioral measures. The procedure for restricting food intake was determined in consultation with LAM husbandry personnel and experienced investigators from other universities. Upon arrival, all rats were fed *ad libitum* and weighed for 3-4 days to confirm that all rats ate normally. To attenuate body weight gains, each experimental rat was given approximately 12 grams of food pellets per day, starting one week before the beginning of the experiment. The free-feeding rats continued to be fed *ad libitum* throughout the duration of the experiments, and their food consumption was recorded daily. All rats' body weight was closely monitored during this time, and when the experimental rats' body weight was within 85-90% of the body weight of the additional rats that were fed *ad libitum*, the amount of daily food given to the experimental rats was increased to maintain body weight within the target weight range. After rats reached the target weight range, their daily food was increased to approximately 75-85% of the amount of food the free-feeding rats had consumed in the previous 24 hours.

Throughout the experiment, rats' body weight was monitored closely and the amount of food given daily was adjusted appropriately to ensure that rats were maintained at approximately 85-90% of the free-feeding body weight for their age and rat strain, as was determined by weighing the free-feeding Fischer and Lewis pairs. During this time, rats were healthy and continued to gain weight, but also were motivated to perform on the operant tasks for a food reward. This practice is a standard procedure in experiments using operant tasks with a food reward (Blondel et al., 2003; Carli, Robbins, Evenden, & Everitt, 1983; Humby, Wilkinson, & Dawson, 2005; van Gaalen et al., 2006) to ensure that animals are sufficiently motivated to work to obtain the food reward. Housing room was maintained at room temperature with 40% humidity and a 12 hr reverse light cycle, with lights off at 7:00 a.m. Because rats are nocturnal animals, maintaining a reverse light cycle caused their active (dark) phase to occur during the daytime, allowing daytime behavioral testing to take place during the rats' active (dark) phase. During testing and handling in the testing and housing rooms, overhead red lights that the albino rats could not detect were used to allow the experimenters to see without disrupting the rats' light cycle. Cages were changed (replaced with clean cages and bedding) once a week. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH, 1996).

*Independent variables*

There were two between-subjects independent variables in the present experiment with two levels: Rat strain (Fischer, Lewis) and Stress (stress, non-stress). Additionally, time was a within-subject independent variable in the present experiment.

*Combined Predatory and Immobilization Restraint Stress*

A combination of the immobilization restraint stress and predatory stress procedures was used to induce stress in a repeated acute fashion in the current experiments. Unpredictable stressors (e.g., whistle, flashing lights) also were used at random intervals to prevent habituation. All stress induction procedures took place in a designated laboratory room separate from the rooms in which behavioral testing was conducted. Varying the stress procedures (restraint stress, fox urine exposure, and unpredictable stimuli) on different stress days was intended to prevent habituation because the rats would not be exposed to the same stressor in the same manner every day. Therefore, varying the stress procedure was intended to increase the likelihood of observing the hypothesized effects of stress and rat strain.

The immobilization restraint stress procedure has long been used as an acute non-painful stressor in our laboratory and others, and produces a reliable increase in corticosterone levels (e.g., Faraday, 2000; Kant, Leu, Anderson, & Mougey, 1987; Kant, Mougey, & Meyerhoff, 1986; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Shaham, Alvares, Nespor, & Grunberg, 1992). The

procedure involves restraining the rats in finger-like devices (Centrap cage) for 20 minutes.

The predatory stress procedure, in which rats are exposed to predator odors, is also an effective stressor that produces a reliable increase in corticosterone levels (Berger, 2009; Campbell, Lin, DeVries, & Lambert, 2003; Hayley, Borowski, Merali, & Anisman, 2001; Long, 2010; Perry, 2009). To induce predatory stress, each rat is exposed for 10 minutes to a cotton ball soaked with 15 ml of commercially available fox urine (Buck Stop Lure Co., Inc., Stanton, MI) in a standard-sized mouse cage (28.5 x 17 x 12 cm) or standard-sized rat cage (42.5 x 20.5 x 20 cm) with a lid in a designated procedure room. The location of the cotton ball within the cage varied each day. In the present experiment, the predatory stress procedure was combined with exposure to unpredictable stimuli (e.g. whistle, flashing lights) to prevent habituation.

Unpredictable stressors, such as flashing lights and loud noises, are also effective stressors that produce reliable increases in corticosterone levels (Berger, 2009; Fride, Dan, Feldon, Halevy, & Weinstock, 1986; Weinstock, Matlina, Maor, Rosen, & McEwen, 1992) in rodent studies. Based on the procedures of Berger (2009) as developed in our laboratory, rats were exposed to unpredictable stimuli at random intervals to prevent habituation to the predatory and immobilization restraint stressors (e.g., Miller et al., 2008; Haile et al., 2001; Ortiz et al., 1996).

In the present experiment, stress induction occurred for four days as depicted in the timeline below (Table 2). On the first day of stress induction, all

rats were immobilized for 20 minutes in restrainers in a separate, designated room that was brightly-lit by overhead fluorescent lights, illuminated at approximately 311.5 lx (Advanced Light Meter, Model Number 840022, Sper Scientific Ltd.). On the second day of stress induction, all rats were exposed to cotton balls soaked with 15 ml of commercially available fox urine in a standard-sized mouse cage (28.5 x 17 x 12 cm) with a lid, and coin-shake at random intervals for 10 minutes in the designated, brightly-lit room. On the third stress day of Experiment One, the two procedures were combined so that during immobilization restraint stress, rats were exposed to cotton balls soaked with 15 ml of commercially available fox urine in a standard-sized rat cage (42.5 x 20.5 x 20 cm) with a lid in the designated, brightly-lit room. On the fourth day of stress induction, rats were again stressed by 20 minutes of exposure to immobilization restraint stress in the designated, brightly-lit room.

Table 2. Experiment 1 Stress Induction Timeline

| Stress Phase Day | Stress Induction Procedure |
|---|---|
| 1 | Restraint Stress |
| 2 | Predator Stress + Coin Shake |
| 3 | Restraint Stress + Predator Stress |
| 4 | Restraint Stress |

*Dependent variables*

There were three dependent variables in the present experiment: impulsive action, body weight, and locomotor activity (to measure general activity and movement). The timelines for Experiments 1 and 2 are provided in

Appendix A. Pictures of the 5-CSRTT and locomotor activity chambers are provided in Appendix B. Data sheets for collection of 5-CSRTT, locomotor, and body weight data are provided in Appendix C.

*Impulsive action and attention*

Impulsive action was measured by premature responses and attention was measured by total omissions and total correct responses in the Five Choice Serial Reaction Time Task (5-CSRTT). More premature responses indicated more impulsivity, and more correct responses and less omissions indicated better attention.

The 5-CSRTT equipment consists of four operant conditioning chambers, each housed in a sound-attenuating box (Med Associates Inc, St. Albans, Vermont, USA). The rear wall of each chamber is a curved metal surface containing a row of five nose-poke apertures. An infra-red photocell beam transverses each aperture to detect nose pokes, and a yellow LED light is fixed at the rear of each aperture. In each chamber, on the opposite wall from the apertures, a pellet dispenser delivers 45 mg pellets (Noyes precision pellets) into a food-hopper. Chamber illumination is provided by a house light located above the food tray. Data collection and presentation of stimuli and rewards is controlled by a computer (Med-PC version 4.0, Med Associates).

Rats were trained on the 5-CSRTT following the procedures of van Gaalen et al. (2006a). In the 5-CSRTT, rats are required to respond to brief flashes of light randomly presented in one of the five apertures by making a nose-poke in the illuminated aperture. Illumination of the house light during each

trial signals that correct responses will be rewarded with a food pellet. Responses that occur during the intertrial interval, when the house light is not illuminated, do not result in food pellet delivery and are recorded as premature responses. Premature responses also are counted when the house light is on and rats make a nose-poke into a hole before a cue light has been illuminated. The total number of premature responses provides an index of impulsive action, such that a greater number of premature responses indicates greater impulsive action.

Table 3. Training Timeline

| Training Week | Training Phase |
|---|---|
| 1 | Gentle/5-CSRTT Acclimation |
| 2 | 5-CSRTT Acquisition Phases 1 and 2 |
| 3 | 5-CSRTT Training Phases 1 and 2 |
| 4 | 5-CSRTT Training Phases 1 and 2 |
| 5 | 5-CSRTT Training Phases 1 and 2 |
| 6 | 5-CSRTT Training Phases 1 and 2 |
| 7 | 5-CSRTT Discrimination Phase |
| 8 | 5-CSRTT Discrimination Phase |
| 9 | 5-CSRTT Discrimination Phase |
| 10 | 5-CSRTT Discrimination Phase |
| 11 | 5-CSRTT Discrimination Phase |
| 12 | 5-CSRTT Discrimination Phase |

Training on the 5-CSRTT consisted of five phases: two acquisition phases, two training phases, and a discrimination phase, as depicted in the training timeline above (van Gaalen et al., 2006a). In the first acquisition phase, rats were allowed to acclimate to the chamber and their behavior was shaped to approach and place their heads inside each of the nose-poke apertures. During the 20-minute sessions, the house light remained on and each of the five nose-

poke apertures contained two food pellets. When animals reliably ate all 10 pellets within a session, training progressed to the next phase. The second acquisition phase was intended to allow the rats to learn to associate the sound of pellet delivery with the food reward. During 25 minute sessions, the house light remained on, and 100 pellets were automatically delivered into the food hopper at an average of 15 second intervals. The second acquisition phase was complete after two sessions.

In the first training phase, rats learned to associate food pellet delivery with an operant nose-poke response. During 30-minute sessions, the house light and all cue lights (the light inside each nose-poke aperture) were illuminated. Food pellet delivery occurred each time rats made a nose-poke response. A session ended after 100 pellets were delivered or 30 minutes had passed. All rats must have reliably earned 100 pellets within 30 minutes to progress to the next phase.

In the second training phase, rats learned that a nose-poke response in an aperture is rewarded only when the cue light inside the aperture is illuminated. The house-light was illuminated during each session of the second training phase. Each trial within a 30-minute session began with the illumination of one of five apertures in pseudorandom order (pseudorandom numbers are uniformly distributed random numbers that are generated by a software function). If the rat responded in the correct hole, then a pellet was delivered into the food hopper. After each correct responses, a 5-second Inter-Trial Interval (I.T.I) began, during which time only the house light was illuminated. The 5-second I.T.I. was followed

by the next trial. Responses into non-illuminated holes were recorded but did not have consequences. After all animals reliably earned 100 pellets within 30 minutes, training progressed to the last phase.

In the discrimination phase of 5-CSRTT training, rats learned to respond exclusively and quickly to cue lights of a progressively shorter duration. Each session started after a 2-minute acclimation period, during which time only the house light was illuminated. Each session terminated after 100 trials occurred or 30 minutes elapsed.

Within a session, each trial began with the illumination of one cue light in pseudorandom order for a maximum of 16 seconds or until a nose-poke response was made. A correct response was counted as one that occurred during stimulus presentation or within a limited hold of 2 seconds after the stimulus light was extinguished. Correct responses were rewarded with food pellet delivery, and followed by extinction of the stimulus light (if necessary) and initiation of a 5-second I.T.I. during which time only the house light was illuminated.

Incorrect responses were counted when responses were made in a non-illuminated hole. Omissions were recorded when a rat did not respond during the cue light illumination or 2 second limited hold. Incorrect responses and omissions were followed by the extinguishment of the stimulus light in the correct hole (if necessary), and punished by a 5 second time-out period, during which time all stimulus lights and the house light were turned off. The 5 second time-out period was followed by a 5 second I.T.I. during which time only the house

light was illuminated. Nose-pokes during an I.T.I. or time-out period resulted in the initiation of a new time-out period.

After nine sessions of training with a 16-second stimulus duration, there was a gradual reduction of stimulus duration (i.e., 16, 8, 4, 2, 1.5, and 1 second). Training was considered complete after rats' performance was stable for at least 5 sessions when a stimulus duration of 1 second was used, with rats reliably making nose-poke responses into apertures at the appropriate times. The training criterion for inclusion in the experiment was that rats had completed all phases of training within 12 – 13 weeks.

The 5-CSRTT provides measures of impulsive action and attention, with both types of measures being collected in the same experimental session. In the 5-CSRTT, total number of premature responses index impulsive action, with more premature responses indicating more impulsive action. Premature responses are responses that occur before a cue-light is illuminated, or during a time-out period. Attention is indexed in the 5-CSRTT by total number of correct responses and total number of omissions, with more correct responses and less omissions indicating better attention.

*Locomotor activity*

Locomotor activity measurements provide information about a rat's pattern of movement in an open field arena (Campbell et al., 2003; Boguszewski & Zagrodzka, 2002; Elliott & Grunberg, 2005; Faraday, 2000; Grunberg, Bowen, &

Morse, 1984; Pare, Blair, Kluczynski, & Tejani-Butt, 1999) and provide an index of behavioral sensitization to psychostimulants, such as nicotine (Vanderschuren & Kalivas, 2000, Robinson & Berridge, 1993, DiFranza & Wellman, 2007). Locomotor activity was measured using electronic physical activity monitoring chambers. Each rat was placed into an individual chamber for one hour to measure open field locomotor activity and record vertical and horizontal movement via a grid of infra-red light beams. Equally spaced beams traverse the plastic arenas (40 x 40 x 30 cm) from front to back and left to right. The body of the rat in the chamber breaks the beams, revealing movement on all parameters collected, including horizontal and vertical movement and center time. The apparatus monitors animal activity continuously for a total testing period of 1 hour, collecting data in 5-minute bins. Dependent variables collected include center time, horizontal activity, and vertical activity. Analyses were performed on total scores for each dependent variable. The main activity-related variable examined was total horizontal activity (Perry, 2009; Berger, 2009; Long, 2010; Starosciak, 2010).

Locomotor activity can be used to provide information about general movement (Hamilton et al., 2009), depression (Faraday, 2002), responses to a novel environment (Campbell et al., 2003), or responses to various stimuli (DiFranza & Wellman, 2007). Locomotor activity measurements were conducted in Experiment 1 to reveal rats' general movement. This information allowed for the determination of whether any differences in performance on the 5-CSRTT were accounted for by differences in general movement.

*Body Weight*

Body weight is often measured in preclinical investigations to provide information about the general health of the rat subjects (e.g., Berger, 2009; Brown & Grunberg, 1995; Elliott, Faraday, Phillips, & Grunberg, 2005; Faraday, 2002; Perry, 2009; Long, 2010; Starosciak, 2010). Additionally, in Experiment 1, body weight data were collected to verify that rodents were kept at 85-90% of free-feeding body weight. This practice is a standard procedure in experiments using operant tasks with a food reward (Blondel et al., 2003; Carli, Robbins, Evenden, & Everitt, 1983; Humby, Wilkinson, & Dawson, 2005) to ensure that animals are sufficiently motivated to work to obtain the reward. To measure body weight, rats were placed individually into a weighing pan on an electronic scale. The electronic scale obtained multiple weight measurements and provided an average of these measurements to increase accuracy and avoid artifacts from movement.

## *Procedure*

*Impulsivity Assessment*

Prior to the beginning of the experiments, all rats received a 12 week training session on the 5-CSRTT, as depicted in the training timeline (Table 3). The experiment began after rats had completed all phases of training and their performance was stable for at least five sessions, as described above. Impulsivity was indexed as premature responses on the 5-CSRTT.

*Attention Assessment*

At the same time that rats were being assessed for impulsivity in the 5-CSRTT, they also were being assessed for attention. To perform correctly on the 5-CSRTT, rats must attend to the nose poke apertures to detect the brief stimulus presentation. A higher number of correct responses, as well as a lower number of errors of omission, indicate better attention. In contrast, lower numbers of correct responses and higher numbers of omissions indicate attentional deficits. The total omissions and total correct responses parameters were used to index attention in the present experiments (Bizarro, Patel, Murtagh, & Stolerman, 2004; Hahn, Shoaib, & Stolerman, 2002; Robbins, 2002).

*Baseline Phase*

All rats were measured in the locomotor activity chambers before the stress phase. Also, rats were measured once in the 5-CSRTT immediately prior to the stress phase.

*Stress Phase*

After stress induction for the stress-group rats, all rats were tested on the 5-CSRTT for three days, with testing occurring immediately following stress induction for the stressed rats. On day 4, rats were tested in the locomotor activity chambers, with testing immediately following stress induction for the stress-group rats.

*Experiment 1 Data Analysis*

*State impulsivity data.* A repeated-measures ANOVA with rat strain and stress group as between-subjects factors and time as a within-subject factor was conducted to compare baseline and stress phase measures of impulsive action in both rat strains and stress groups. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs. Post hoc tests, when necessary, were Tukey's HSD tests. Tests were two-tailed with $\alpha$ level = 0.05.

*Locomotor activity data.* Locomotor data were analyzed with repeated-measures analyses of variance (ANOVA) with impulsivity group and stress-group as the between-subjects factors, and time as the within-subject factor. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs. Tests were two-tailed with $\alpha$ level = 0.05.

*Attention data.* Attention data were analyzed with repeated-measures analyses of variance (ANOVA) with rat strain and stress group as the between-subjects factors, and time as the within-subject factor. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs. Tests were two-tailed with $\alpha$ level = 0.05.

*Sample size.* As discussed earlier in the power analysis, an N of 32 rats was used in Experiment 1, with 8 rats per cell. This sample size yielded sufficient power to detect main effects of stress and rat strain with the effect size

observed in the experiment by Kearns et al. (2006), in which measures related to impulsive action were conducted.

# RESULTS

## Experiment 1: The Effect of Stress on State Impulsivity and Attention In Lewis and Fischer Rats

The findings for Experiment 1 are reported below. First, the data analytic strategy is outlined. Presentation of the experimental findings organized by dependent variable follows, in the order of impulsive action results, attention results, and locomotor activity results, respectively. The findings are illustrated in graphs interspersed throughout the results section text, and the statistics supporting each finding are presented in tables in Appendix D.

### *Experiment 1: Data Analytic Strategy*

*State impulsivity data*. The premature responses parameter on the 5-CSRTT was used to index impulsive action. A two-way ANOVA was conducted with stress and rat strain as the between-subjects factors to determine whether differences existed in premature responses at baseline between groups. To account for baseline differences, baseline premature responses were used as a covariate in subsequent analyses of premature responses. A repeated-measures ANCOVA with rat strain and stress group as between-subjects factors, time as a within-subject factor, and baseline impulsive action as the covariate was conducted to compare stress phase premature responses in both rat strains and stress groups. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANCOVAs and additional repeated-measures ANCOVAs with baseline premature responses as the covariate. Tests were two-tailed with $\alpha$ level = 0.05.

*Attention data.* The correct responses and omissions parameters on the 5-CSRTT were used to index attention. A two-way ANOVA was conducted with stress and rat strain as the between-subjects factors to determine whether differences existed in correct responses at baseline between groups. Repeated-measures ANOVAs with rat strain and stress group as between-subjects factors and time as a within-subject factor were conducted to compare stress phase correct responses and omissions in both rat strains and stress groups. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs and additional repeated measures ANOVAs. Tests were two-tailed with $\alpha$ level = 0.05.

*Locomotor activity data.* Locomotor data were analyzed with repeated-measures analyses of variance (ANOVA) with impulsivity group and stress group as the between-subjects factors, and time as the within-subject factor. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs and repeated-measures ANOVAs. Tests were two-tailed with $\alpha$ level = 0.05.

## Experiment 1: Results

### *State Impulsivity*

*Baseline Day.* Lewis rats had more premature responses than Fischer rats at baseline [$F(1, 30) = 7.955$, $p < 0.01$]. Therefore, Lewis rats had greater impulsive action than did Fischer rats at baseline. The baseline impulsive action

results are displayed in Figure 4, and the statistics are presented in Table 1A of Appendix D.

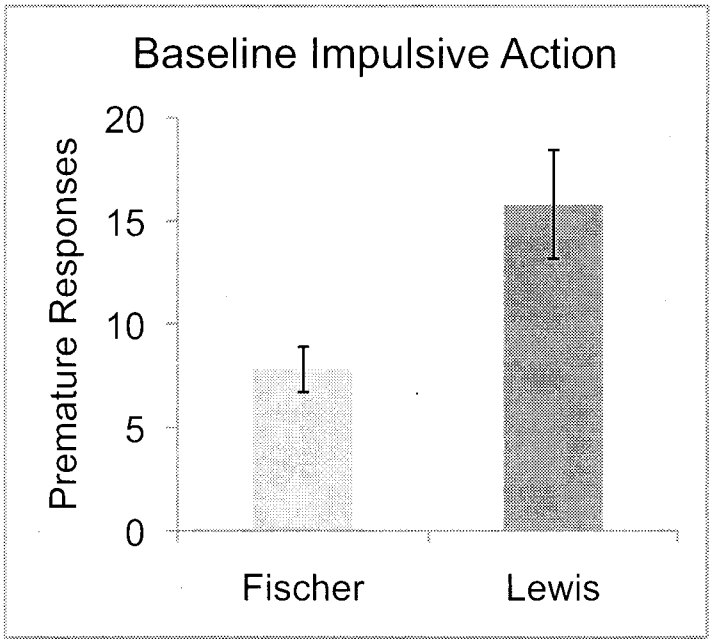



Figure 4. Baseline impulsive action in Fischer and Lewis rats [mean ± Standard Error of the Mean (S.E.M.)]

Lewis rats had more premature responses than Fischer rats in the non-stress group [$F(1, 14) = 4.861$, $p < 0.05$]. Corresponding statistics are presented in Tables 1B and 1C of Appendix D.

*All stress days.* Overall, Lewis stressed rats had fewer premature responses than did Lewis non-stressed rats, and Fischer stressed rats had more premature responses than did Fischer non-stressed rats [$F(1,27)= 7.689$, $p = 0.010$]. Stress decreased impulsive action (premature responses) in Lewis rats and increased impulsive action in Fischer rats. The premature response results

for all experimental days are displayed in Figures 5 and 6, and the corresponding statistics are presented in Table 2A of Appendix D.

Stressed Lewis rats had fewer premature responses than did non-stressed Lewis rats [$F(1,13) = 23.602$, $p < 0.001$]. Stressed Fischer rats had greater premature responses than did stressed Lewis rats [$F(1,13)=5.377$, $p < 0.05$]. Corresponding statistics are presented in Tables 2B and 2C of Appendix D.

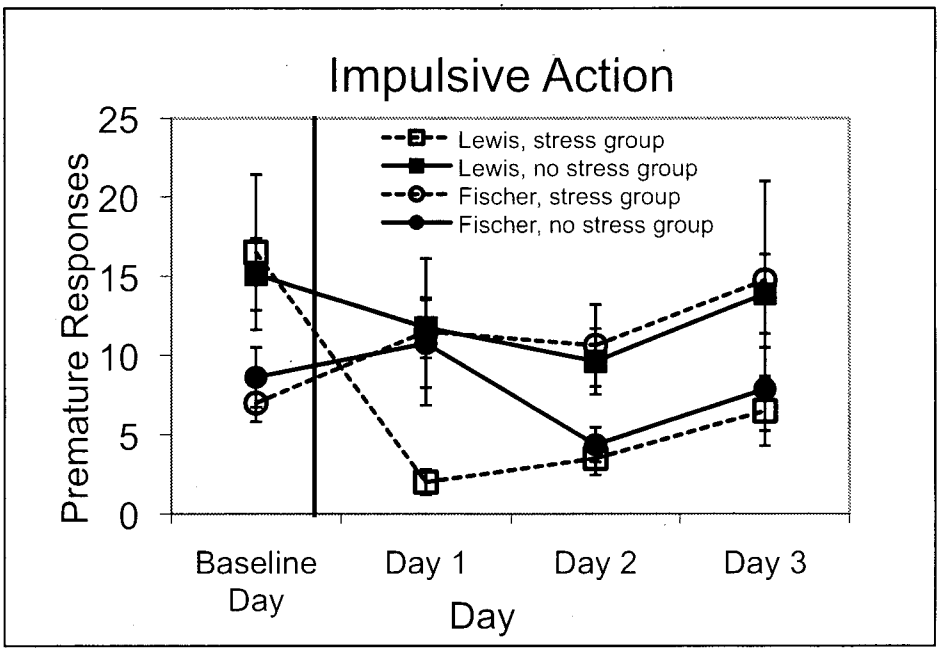



Figure 5. Premature responses across all experimental days (mean ± S.E.M.)

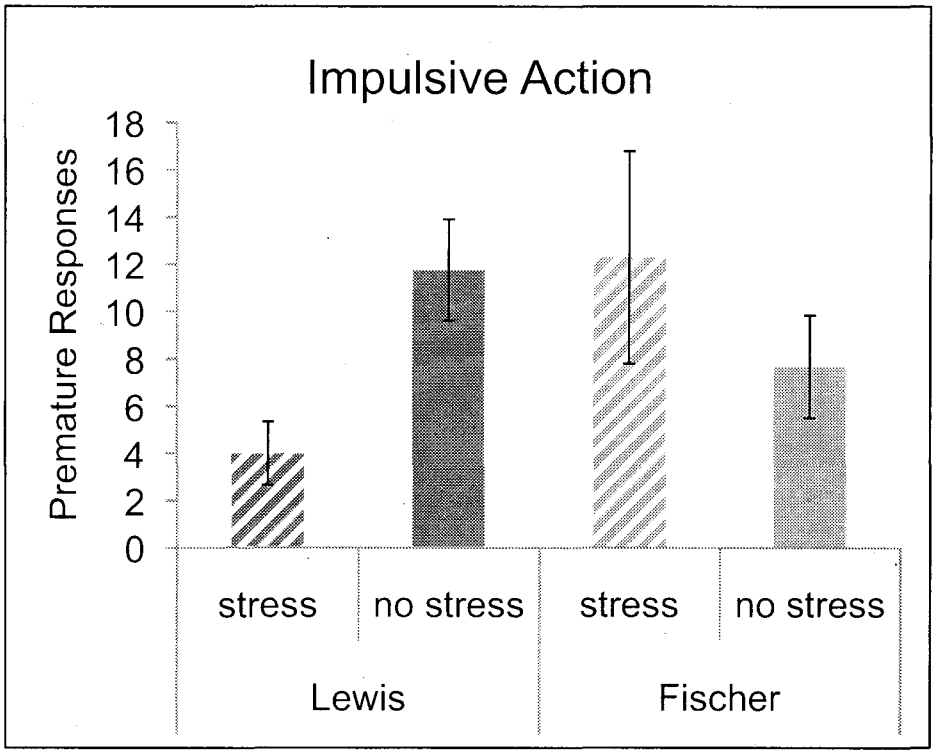



Figure 6. Premature Responses in stress-group and non-stress-group Lewis and Fischer rats, averaged across days 1, 2, and 3 (mean ± S.E.M.).

*Summary: Impulsive Action.* Lewis rats had more premature responses than did Fischer rats at Baseline. These results indicate that Lewis rats have greater impulsive action than Fischer rats without stress. Across all stress days, stress decreased impulsive action in Lewis rats and increased impulsive action in Fischer rats.

*Confirmation of hypotheses: Impulsive Action.* **The hypothesis that Lewis rats would perform more impulsively than Fischer rats at baseline (Hypothesis 1A) was confirmed.** Lewis rats had significantly more premature responses than Fischer rats at the baseline measurement. **The hypothesis that**

**stress would increase impulsive action in Lewis and Fischer rats (Hypothesis 1B) was partially confirmed.** Stress increased impulsive action in Fischer rats, but decreased impulsive action in Lewis rats. **The hypothesis that effects of stress to increase impulsive action would be greater in Lewis rats than Fischer rats (Hypothesis 1C) was not confirmed.** The effect of stress to increase impulsive action occurred exclusively in Fischer rats; stress decreased impulsive action in Lewis rats.

*Attention*

*Baseline Day: Correct Responses.* Lewis and Fischer rats did not have significantly different amounts of correct responses at the baseline measurement. Baseline correct responses results are displayed in Figure 7, and corresponding statistics are presented in Table 3A in Appendix D.

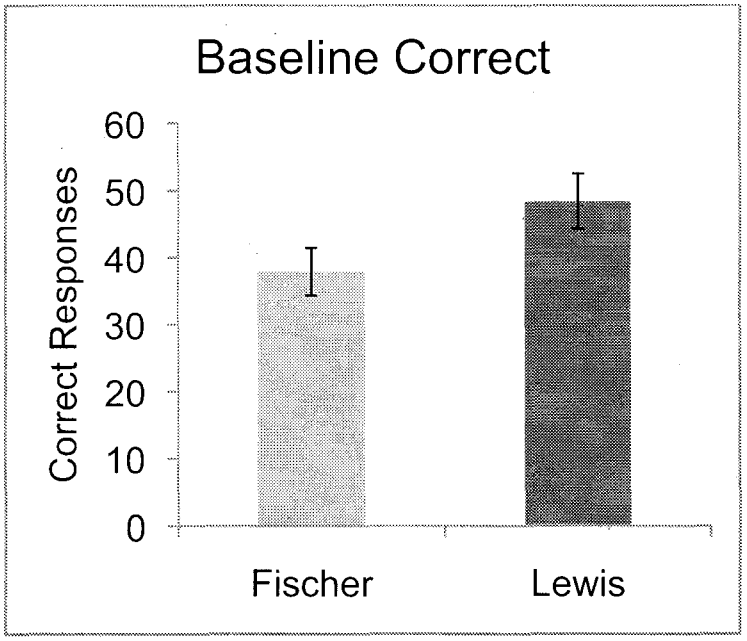



Figure 7. Baseline correct responses in Lewis and Fischer rats (mean ± S.E.M.).

*Baseline Day: Omissions.* Amount of omissions did not differ between Lewis and Fischer rats. Baseline omissions results are displayed in Figure 8, and corresponding statistics are presented in Table 3B in Appendix D.

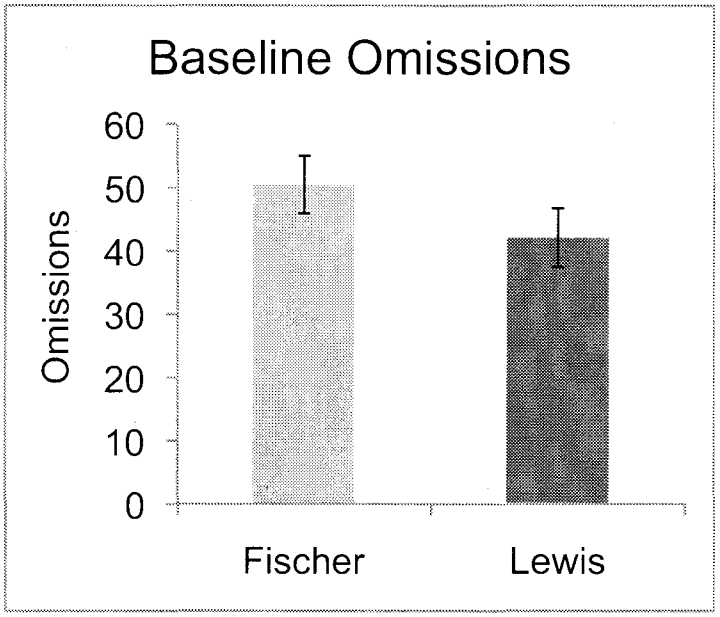



Figure 8. Baseline omissions in Lewis and Fischer rats (mean ± S.E.M.).

*All stress days: Correct Responses.* The effects of stress on correct responses changed across days [$F(2,56) = 5,780$, $p < 0.01$]. Non-stress-group rats had more correct responses than did stress group rats [$F(1,28) = 67.748$, $p < 0.001$]. Non-stress group Lewis rats had more correct responses than did non-stress group Fischer rats, and stress group Lewis rats had less correct responses than did stress group Fischer rats [$F(1,28) = 7.435$, $p < 0.05$]. The present data are displayed in Figures 9 and 10, and the corresponding statistics are presented in Tables 4A, 4B, and 4C.

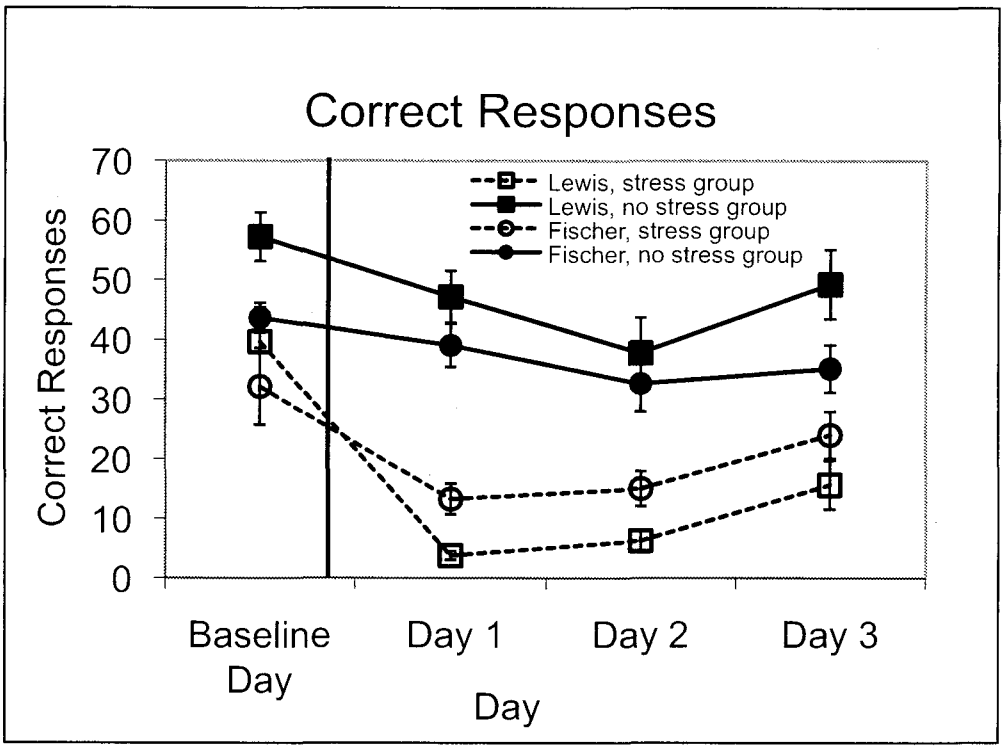



Figure 9. Correct responses across all experimental days in stress-group and non-stress-group Lewis and Fischer rats (mean ± S.E.M.).

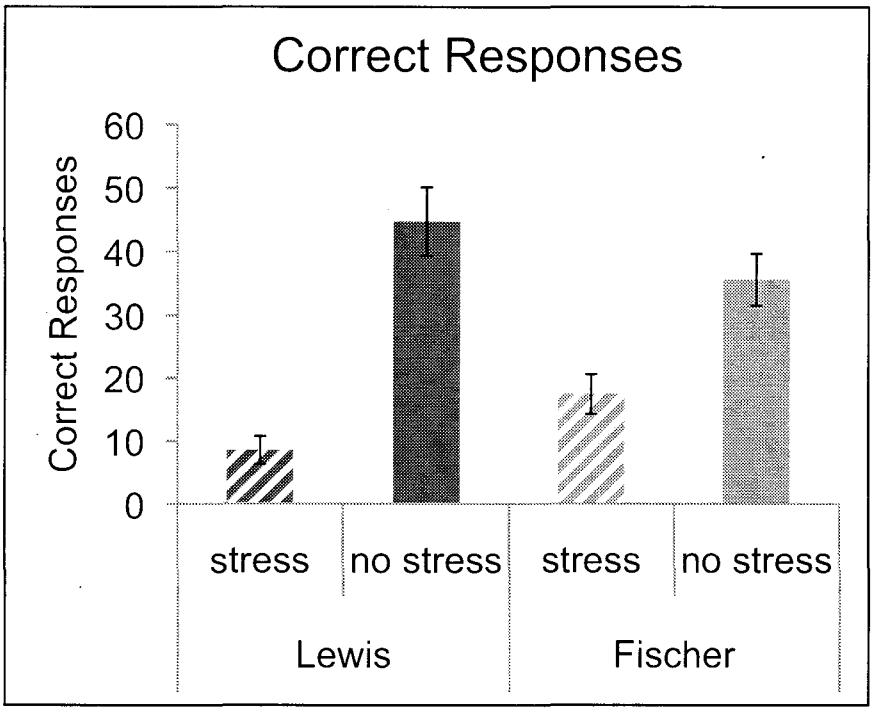



Figure 10. Correct Responses in stress-group and non-stress-group Lewis and Fischer rats, averaged across days 1, 2, and 3 (mean ± S.E.M.).

Non-stress group Lewis rats had more correct responses than did stress-group Lewis rats [$F(1,14) = 58.523$, $p < 0.001$]. In Fischer rats, effects of stress on correct responses varied across days [$F(2,28) = 6.183$, $p < 0.01$]. Non-stress-group Fischer rats had more correct responses than did stress-group Fischer rats [$F(1,14) = 15.550$, $p < 0.001$]. Additionally, Fischer stressed rats had more correct responses than did Lewis stressed rats [$F(1,14) = 7.511$, $p < 0.05$]. Correct Responses across all stress days are displayed in Figures 9 and 10, and corresponding statistics are presented in Tables 4A, 4B, and 4C.

*All stress days: Omissions.* Effects of stress on omissions varied across days [$F(2,56) = 5.427$, $p < 0.01$]. Stress-group rats had more omissions than did non-stress-group rats [$F(1,28) = 32.866$, $p < 0.001$]. Lewis rats had the most omissions of the stressed rats and the least omissions of the non-stressed rats [$F(1,28) = 7.630$, $p < 0.05$]. The present data are displayed in Figures 11 and 12, and corresponding statistics are presented in Tables 4D in Appendix D.

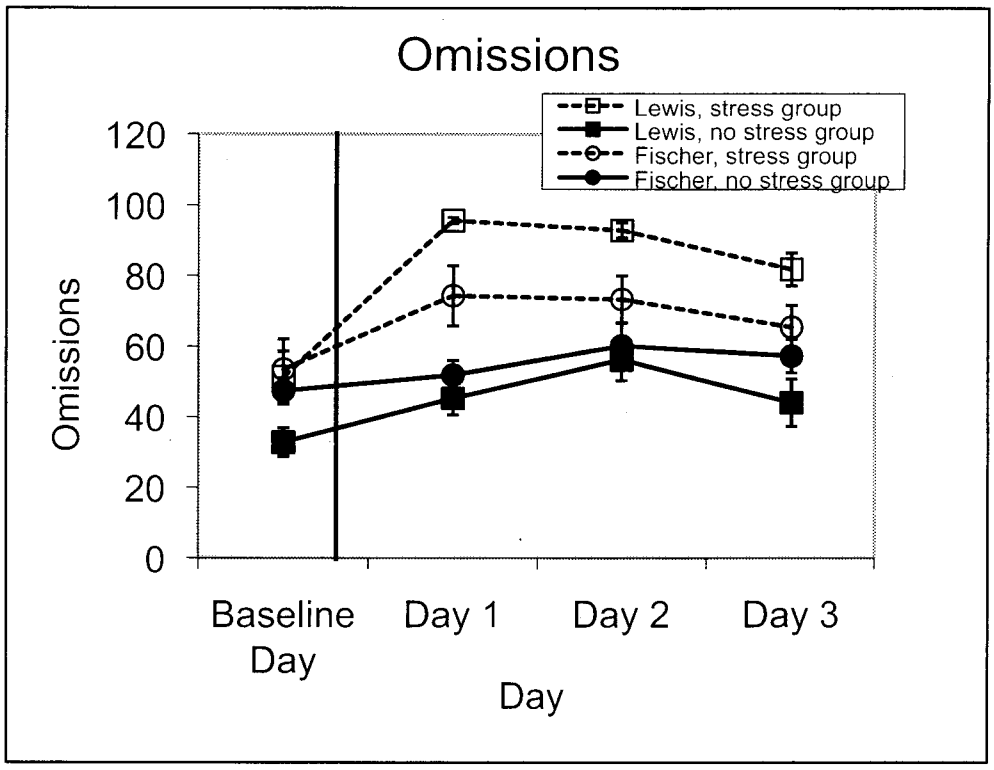



Figure 11. Omissions across all experimental days in stressed and non-stressed Lewis and Fischer rats (mean ± S.E.M.).

Omissions varied across stress days in Lewis rats [$F(2,28) = 5.334$, $p < 0.05$]. Stressed Lewis rats had more omissions than did non-stressed Lewis rats. [$F(1,14) = 67.394$, $p < 0.001$]. In Fischer rats effects of stress on omissions varied across days [$F(2,28) = 4.168$, $p < 0.05$]. Additionally, Lewis stressed rats had more omissions than did Fischer stressed rats [$F(1,14) = 7.270$, $p < 0.01$]. Corresponding statistics are presented in Tables 4E and 4F.

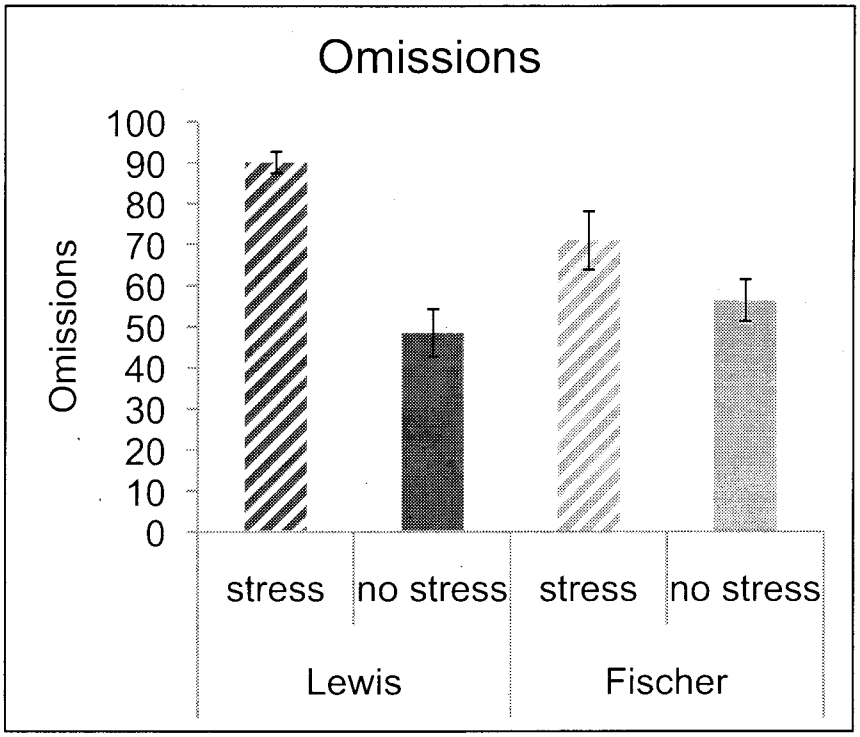



Figure 12. Omissions in stress-group and non-stress-group Lewis and Fischer rats, averaged across days 1, 2, and 3 (mean ± S.E.M.).

*Summary: Attention.* There were no significant differences between rat strains on the omissions or correct responses parameters at baseline. Stress decreased attention in Lewis and Fischer rats on both parameters. An interaction occurred on both parameters, such that non-stressed Lewis rats had the best attention compared with Fischer rats, whereas stressed Lewis rats had the worst attention compared with Fischer rats. These effects occurred on each individual stress day, as well as when all stress days were considered together.

*Confirmation of Hypothesis: Attention.* **It was hypothesized that attention would be decreased in Lewis rats compared with Fischer rats**

**(Hypothesis 1D).** This hypothesis was **partially confirmed.** Stressed Lewis rats had decreased attention compared to stressed Fischer rats. However, non-stressed Lewis rats had better attention than non-stressed Fischer rats.

*Correlations: Impulsive Action and Attention*

Bivariate correlations were conducted to determine the correlations between premature responses and correct responses, and premature responses and omissions, on each day that the 5-CSRTT was used in Experiment 1. On days in which correlations were significant, the data were further split by stress and rat strain and additional correlations were conducted.

*Baseline Day.* On baseline day, premature responses were positively correlated with correct responses [$r = 0.423$, $p < 0.05$] and negatively correlated with omissions [$r = -0.507$, $p < 0.01$]. When the file was split by rat strain, correlations between premature responses and correct responses were not significant in either rat strain. Correlations between premature responses and omissions were significant in Lewis rats [$r = -0.507$, $p < 0.05$] but were not significant in Fischer rats. When the file was split by stress group (although stress was not induced on baseline day), premature responses and correct responses were significantly positively correlated in the stress group [$r = 0.499$, $p < 0.05$], but not in the non-stress group. Premature responses and omissions were significantly negatively correlated in both the stress group [$r = -0.499$, $p <$

0.05] and the non-stress group [$r$ = -0.636, $p < 0.01$]. The results are presented in statistics tables 5A, 5B, and 5C of Appendix D.

*Stress Day 1.* On stress day 1, premature responses were correlated positively with correct responses [$r$ = 0.382, $p < 0.05$] and negatively with omissions [$r$ = -0.416, $p < 0.05$]. When the file was split by rat strain, premature responses and correct responses [$r$ = -0.789, $p < 0.01$] and premature responses and omissions [$r$ = -0.804, $p < 0.01$] were negatively correlated in Lewis rats only. When the data file was split by stress group, premature responses and correct responses were significantly negatively correlated in the stress group [$r$ = -0.859, $p < 0.01$]. When the file was split by rat strain and stress group, premature responses were positively correlated with correct responses in Fischer stressed rats [$r$=0.853, $p < 0.01$]. The results are displayed in Tables 6A, 6B, and 6C of Appendix D.

*Stress Day 2.* On stress day 2, premature responses were significantly correlated with neither correct responses nor omissions. However, when the file was split by rat strain and stress group, premature responses and correct responses were positively correlated in Lewis stressed rats [$r$=0.858, $p < 0.01$] and Fischer stressed rats [r=0.856, $p < 0.01$], and premature responses and omissions were negatively correlated in Lewis stressed rats [r=-0.898, $p < 0.01$]. The results are presented in Table 7A of Appendix D.

*Stress Day 3.* On stress day 3, premature responses were significantly positively correlated with correct responses [$r = 0.350$, $p < 0.05$] and negatively correlated with omissions [$r = -0.470$, $p < 0.01$]. When the file was split by rat strain, premature responses were positively correlated with correct responses [$r = 0.723$, $p < 0.01$] and negatively correlated with omissions [$r = -0.742$, $p < 0.01$] in Lewis rats only. When the data file was split by stress group, correlations between premature responses and correct responses [$r = 0.580$, $p < 0.05$] and premature responses and omissions [$r = -0.744$, $p < 0.01$] were only significant in the non-stress group. When the data file was split by stress and rat strain, premature responses and omissions were negatively correlated in Fischer non-stressed rats [$r=-0.751$, $p < 0.05$]. The results are displayed in Tables 8A, 8B, and 8C of Appendix D.

*Summary: Impulsive Action and Attention Correlations.* Measures of impulsive action and attention were correlated on Baseline Day, Stress Day 1, Stress Day 2, and Stress Day 3. Almost all the correlations occurred in the Lewis group and not in the Fischer group. On Stress Days 1 and 2, correlations occurred in the stress group, while on Stress Day 3 correlations occurred in the non-stress group.

*Locomotor activity.* Locomotor activity did not differ among stress and rat strain groups in Experiment 1. The lack of group differences in locomotor activity suggests that group differences in measures of impulsive action and attention on

the 5-CSRTT were not accounted for by any group differences in overall general movement. Experiment 1 locomotor activity data are displayed in Figure 13, and are presented in Table 9A of Appendix D.

*Summary: Locomotor Activity.* There were no group differences in locomotor activity in Experiment 1, indicating that effects of stress and rat strain on measures of impulsive action and attention were not influenced by differences in general movement.

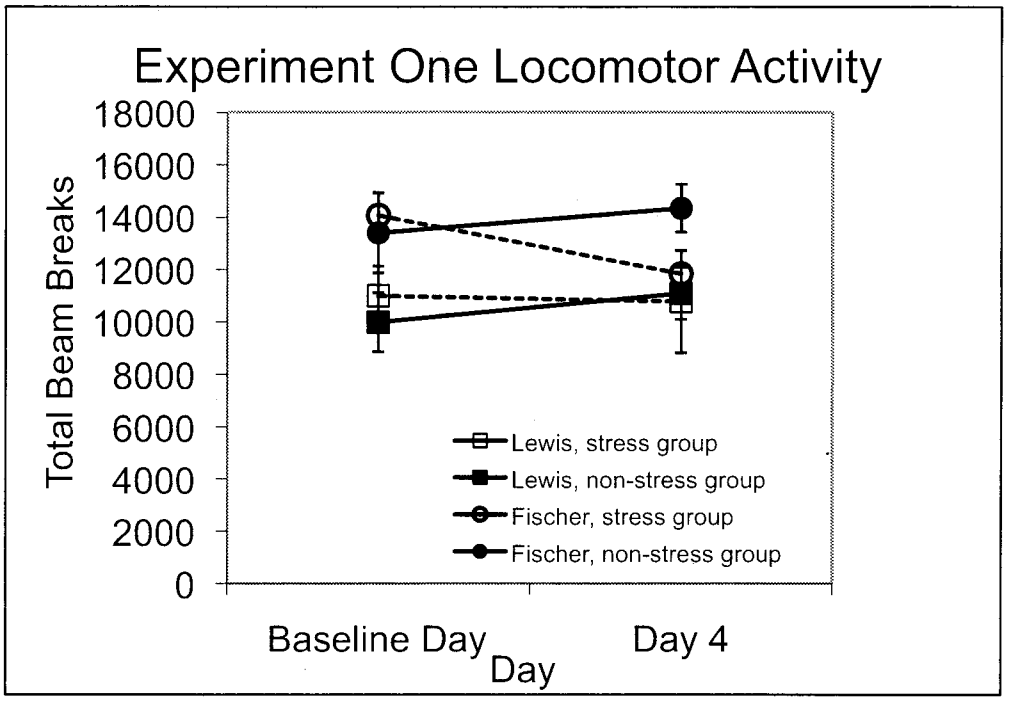



Figure 13. Experiment 1 locomotor activity in stress-group and non-stress-group Lewis and Fischer rats (mean ± S.E.M.).

*Experiment 1 Summary:* As depicted in Table 4, effects of stress differed in Lewis and Fischer rats on most of the dependent variables measured in Experiment 1. Impulsive action was decreased in Lewis stressed rats compared

to Lewis non-stressed rats, but impulsive action was increased in Fischer stressed rats compared to Fischer non-stressed rats. Lewis non-stressed rats and Fischer stressed rats had roughly equal levels of impulsive action, the two groups had more impulsive action than Fischer non-stressed rats, and Lewis stressed rats had the lowest level of impulsive action. Stressed rats had fewer correct responses than non-stressed rats in both the Lewis and Fischer rat strains. Lewis non-stressed rats had more correct responses than Fischer non-stressed rats, which had more correct responses than Fischer stressed rats. Lewis stressed rats had the fewest correct responses. Stressed rats had more omissions than non-stressed rats in both rat strains. Lewis stressed rats had more omissions than Fischer stressed rats, which had more omissions than Fischer non-stressed rats, which had more omissions than Lewis non-stressed rats. Horizontal activity did not differ significantly among the stress and rat strain groups.

| Dependent Variable | Lewis | Fischer | Lewis vs. Fischer |
|---|---|---|---|
| **Impulsive Action** | S < NS * | S > NS * | LNS = FS > FNS > LS * |
| **Correct Responses** | S < NS * | S < NS * | LNS > FNS > FS > LS * |
| **Omissions** | S > NS * | S > NS * | LS > FS > FNS > LNS * |
| **Horizontal Activity** | S = NS | S < NS | FNS > LNS = FS = LS |

Table 4. Experiment 1 Effects. S indicates stress group, NS indicates non-stress group, L indicates Lewis rats, F indicates Fischer rats, and * indicates significance at $p < 0.05$.

# EXPERIMENT 1: ASSESSMENT AND DISCUSSION

## Assessment of Experiment 1 Hypotheses

**Hypothesis 1A**: Impulsivity. Lewis rats will perform more impulsively than Fischer rats on the 5-CSRTT: **supported.** Lewis rats initially had more premature responses than Fischer rats on the 5-CSRTT, indicating greater impulsivity.

**Hypothesis 1B:** Stress and Impulsivity. Psychological stress will increase impulsive action in Lewis and Fischer rats: **partially supported.** Psychological stress increased premature responses in Fischer rats but decreased premature responses in Lewis rats, indicating that stress increased impulsivity in Fischer rats but decreased impulsivity in Lewis rats.

**Hypothesis 1C**: Stress and Impulsivity in Lewis rats. The effect of stress to increase impulsive action will be greatest in Lewis rats: **not supported.** Stress decreased premature responses in Lewis rats, indicating that stress decreased impulsive action in Lewis rats. Opposite from what was hypothesized, an effect of stress to increase impulsive action occurred in Fischer rats only. However, while the effect of stress on impulsive action in Lewis rats was opposite the hypothesized direction, the effect occurred the most consistently in the Lewis rat strain.

**Hypothesis 1D:** Attention. Lewis rats will have decreased attention compared with Fischer rats on the 5-CSRTT: **not supported.** At baseline, Lewis

and Fischer rats' responses did not differ significantly on the correct responses or omissions parameters.

# DISCUSSION

## Experiment 1: The Effect of Stress on State Impulsivity in Fischer and Lewis Rats

The goal of Experiment 1 was to determine the effect of psychological stress on impulsive action and attention in Fischer and Lewis rats, using the Five-Choice Serial Reaction Time Task (5-CSRTT). The independent variables were psychological stress (stress, non-stress) and rat strain (Fischer, Lewis). Thirty-two male rats (n=8) were tested in a 2 (stress) x 2 (rat strain) factorial design with repeated measures. Rats were initially tested in the 5-CSRTT without stress induction, and then were tested in the 5-CSRTT for three days after stress induction. The dependent variables in the present research were impulsive action, attention, and locomotor activity.

There were several major findings: (1) Lewis rats initially had more impulsive action than Fischer rats; (2) Stress affected impulsive action differentially in both rat strains, with stress decreasing impulsive action in the Lewis rat strain and increasing impulsive action in the Fischer rat strain; (3) Lewis rats initially had better attention than Fischer rats; (4) Stress decreased attention in both rat strains, with a greater effect in the Lewis rats; (5) Impulsive action and attention were correlated. Each of these findings is discussed in detail below.

Consideration is given to relevant methodological issues and limitations of the present research.

**Finding #1: Lewis rats initially had more impulsive action than Fischer rats.** On the baseline day, when rats were measured in the 5-CSRTT without stress induction, Lewis rats had more premature responses than Fischer rats, indicating greater impulsive action. This is a new finding as this is the first experiment to compare Lewis and Fischer rats on a measure of impulsive action, such as the 5-CSRTT. This finding indicates the value of Lewis rats as an animal model of impulsivity.

*Lewis and Fischer Rat Strain Differences*

This research is the first to compare impulsive action in Lewis and Fischer rats. The finding that Lewis rats have more impulsive action than Fischer rats is consistent with the report of greater impulsive choice in Lewis rats compared with Fischer rats (Anderson & Woolverton, 2005). The finding also is consistent with the report of greater sign-tracking in Lewis rats compared with Fischer rats (Kearns et al., 2006), a measure that is thought to be related to impulsivity (Monterosso & Ainslie, 1999; Tomie, Aguado, Pohorecky, & Benjamin, 1998; Winstanley et al., 2004). Sign-tracking, or autoshaping, occurs when an organism approaches and contacts conditioned stimuli that signal unconditioned appetitive stimuli; and has been found to be predicted by impulsivity (Tomie et al, 1998). Previous research reported that Lewis rats are more impulsive than Fischer rats on a measure of impulsive choice (Anderson & Woolverton, 2005) and a measure related to impulsivity, sign-tracking (Kearns et al., 2006). The

present finding, that impulsive action is greater in Lewis rats than Fischer rats, is consistent with these reports and provides further validation of Lewis rats as an animal model of impulsivity.

**Finding #2: Stress affected impulsive action differentially in both rat strains, with stress decreasing impulsive action in the Lewis rat strain and increasing impulsive action in the Fischer rat strain.** On Stress Day 1, when stress was induced using immobilization restrainers, stress decreased impulsive action in Lewis rats, but there was no effect of stress in Fischer rats. On Stress Day 2, when stress was induced by exposure to a predator odor, stress decreased impulsive action in Lewis rats and increased impulsive action in Fischer rats. On Stress Day 3, when stress was induced by a combination of predator odor exposure and immobilization restraint, stress significantly decreased impulsive action in Lewis rats, but the increase in impulsive action in Fischer rats was not significant. The finding that stress affected impulsive action in the 5-CSRTT in Lewis and Fischer rats is new, because the effect of stress on impulsive action has not been examined using the 5-CSRTT and has not been examined in Lewis and Fischer rats.

*Effect of Stress on Impulsivity*

In an unpublished masters thesis, Mahoney (2009) reported no effect of restraint stress on impulsive choice and impulsive action in Long Evans rats using the Delayed Reinforcement and Go/No-Go tasks, respectively. The findings of the present research differ from the findings of Mahoney (2009). The Go/No-go task used by Mahoney measures ability to inhibit a behavior, but

requires a subject to learn that responding is reinforced in the presence of a specific stimulus (Mitchell, 2004). The 5-CSRTT was used in the present research to allow for the assessment of impulsivity without the increased cognitive load of attention, conditional associative learning, and working memory associated with the Go/No-go task (Finn, Justus, Mazas, & Steinmetz, 1999; Finn, Mazas, Justus, & Steinmetz, 2002; Mitchell, 2004).

The stress paradigm used by Mahoney (2009), 1 hour of immobilization restraint stress, is most comparable to Stress Day 1 in the present research, in which stress was induced with 20 minutes of immobilization restraint stress. Mahoney found no effect of 1 hour of restraint stress on impulsive action in Long Evans rats, while 20 minutes of restraint stress caused an effect of stress in Lewis rats, but not in Fischer rats, in the present research. There are several possible explanations for the discrepancies in the research findings.

First, use of the 5-CSRTT is preferable to the Go/No-go task to examine effects of stress on impulsive action because of the increased cognitive load associated with the Go/No-go task (e.g., Finn et al., 2002, 2004; Mitchell, 2004). Effects of stress on cognitive variables such as conditional associative learning and working memory associated with the Go/No-go task may have obscured effects of stress on impulsive action in Mahoney's research. Second, because rat strain differences in effects of stress on impulsive action occurred in the present research with Lewis and Fischer rats, it is possible that Long Evans rats are not susceptible to effects of stress on impulsive action. Differences in HPA-axis reactivity to immobilization restraint stress induction have been reported in

female, but not male, Long Evans rats (Faraday, Blakeman, & Grunberg, 2005). Third, research from the Grunberg laboratory suggests that 20 minutes of restraint stress may be optimal for inducing a stress response (e.g., Shaham, Alvares, Nespor, & Grunberg, 1992). Longer duration of stress (i.e., 1 hour) may cause habituation to the stressor. It is possible that one hour of restraint stress, such as that used by Mahoney (2009), is less effective than 20 minutes of restraint stress in inducing a stress response, and producing an effect on impulsive action.

Interestingly, in the present research, while the main effect of stress happened in Lewis rats on all three stress days, the main effect of stress occurred in the Fischer rats on Stress Day 2 only. On Stress Day 2, stress was induced by predator odor exposure, while stress was induced on Stress Day 1 using restraint stress and Stress Day 3 using combined restraint and predator stress. It is possible that Lewis rats are more sensitive to effects of stress on impulsive action, and that something about the restraint stress procedure dampened the effect of stress on impulsive action in Fischer rats. Because Mahoney (2009) reported no effects of immobilization restrainers on impulsive action, and there were no effects of immobilization restrainers on Fischer rats in the present research, it is possible that stress induction procedures involving immobilization restrainers are less effective at producing effects on impulsive action than stress induction procedures using predator stress alone. Research examining HPA axis responses to predator and restraint stress in rats of various strains is needed to compare the efficacy of the two stress induction techniques.

That the effect of stress on impulsive action occurred more consistently in the Lewis group as compared to the Fischer group is surprising in light of differences in HPA axis responsivity between the two rat strains. Fischer rats are hyper-reactive to stress, while Lewis rats are hypo-reactive to stress (Chaouloff et al., 1995; Dhabar et al., 1993). Fischer rats have higher baseline levels of corticosterone than Lewis rats, and have a greater HPA axis response to an acute stressor. However, in the present research, effects of stress on impulsive action occurred most consistently in the Lewis rat strain. Lewis rats were more impulsive than Fischer rats at baseline. It is possible that stress decreases impulsive action in impulsive individuals (i.e., Lewis rats) regardless of stress reactivity.

*Corticoptrophin Releasing Factor and Impulsivity*

The present findings are inconsistent with a report in which administration of corticotrophin releasing factor (CRF) intracerebroventricularly did not affect impulsivity in Lister hooded rats and Wistar rats on the five-choice serial reaction time task (Ohmura, Yamaguchi, Togashi, Izumi, Matsumoto, Yoshida, & Yoshioka, 2009). Although the authors did not induce stress *per se*, release of CRF is a component of the stress response. There are several possible reasons for the inconsistent results. First, it is possible that CRF is not responsible for any effects of stress on impulsive action. Other components of the rat HPA axis stress response, such as Adrenocorticotrophin Hormone (ACTH) or corticosterone, may play a larger role in effects of stress on impulsive action. Alternatively, effects of stress on the Sympathetic Nervous System (SNS) could

mediate any effects of stress on impulsive action. Because rat strain differences in effects of stress on impulsive action were found in the present research, it is possible that the Lister hooded and Wistar rat strains are not susceptible to effects of stress on impulsive action.

*Stress Reactivity and Attention Deficit Hyperactivity Disorder*

Attention-Deficit Hyperactivity Disorder (ADHD) is a disorder of sustained attention, distractibility, impulse control, and hyperactivity (DSM-IV-TR, 2000). Because impulse control and attention are a focus of the present research, literature examining stress in ADHD is relevant to interpret the results. Reports of differential stress reactivity in people with ADHD are mixed. It has been reported that adults with ADHD had higher levels of subjective stress and reported higher levels of daily stressors than non-ADHD controls, and that higher cortisol levels after a stressor were related to higher levels of impulsivity (Hirvikoski et al., 2009). Inducing psychological stress in ADHD and non-ADHD control adults leads to elevated levels of subjective stress in ADHD adults, and mixed physiological responses (Lackschewitz, et al., 2008). However, it also has been reported that HPA axis under reactivity was associated with impulsivity in boys with ADHD (Hong, et al., 2003). Additionally, children with ADHD had lower levels of epinephrine in response to a challenge (Hanna, et al., 1996) as well as reduced salivary cortisol levels (Kariyawasam et al., 2002), and it has been reported that blunted stress responses may be a marker for a more developmentally pervasive form of ADHD (King et al., 1998). Therefore, some

type of stress dysregulation may be implicated in ADHD, though the direction of the dysregulation is unclear. Research on the association between impulsivity and stress reactivity is needed in individuals without ADHD, in order to rule out effects of stress caused by impairments associated with the disorder.

In the present research, stress reactivity was lowest in the initially impulsive Lewis rat strain, and stress reactivity was highest in the non-impulsive Fischer rat strain. These findings are consistent with the research of Hong et al. (2003).

### *Stress and Reaction Time Variability*

Lee, Shin, and Stein (2010) reported that increased cortisol levels after stress were associated with increased variability in reaction time on the Continuous Performance Test (CPT). However, in their experiment, psychological testing was used to induce stress, a technique that may not have induced an adequate level of stress. Despite this possible limitation, there is a parallel between the report of Lee et al. (2010) and the current research. A stressor increased variability in response time in the Lee et al. (2010) study, and in the present research, stress caused variability of premature responses in two distinct directions—increasing premature responses in the Fischer genetic rat strain and decreasing premature responses in the Lewis genetic rat strain.

*The Role of Baseline Impulsivity*

In the present results, effects of stress on impulsive action differed in two rat strains with differing levels of baseline impulsive action. Stress decreased impulsive action in the initially more impulsive rat strain (Lewis) and increased impulsive action in the initially less impulsive rat strain (Fischer). These results suggest that when examining the effects of a manipulation on impulsivity, it is important to take initial level of impulsivity into account.

The present results are consistent with research in which effects of cocaine (Anker, Zlebnik, Giddon, & Carroll, 2009) and caffeine and d-amphetamine (Barbelivien, Billy, Lazarus, Kelche, & Majchrzack, 2007) on measures of impulsivity differed in rat groups with different levels of baseline impulsivity. Anker and colleagues (2009) reported that prior cocaine exposure increased impulsivity on a delay-discounting task in rats that initially had low levels of impulsivity, while level of impulsivity was unaffected in rats that initially had high levels of impulsivity. Barbelivien and colleagues (2007) separated rats into high, medium, and low impulsivity groups based on their performance on a delay discounting task. Administration of caffeine or d-amphetamine decreased impulsive choice in the medium-impulsivity group only.

In both experiments, effects of manipulations differed as a function of baseline impulsivity. The pattern of results in the research by Anker and colleagues (2009) is especially striking in its similarity to the present results. Rats were divided into two groups--high and low baseline impulsivity—and were tested on a measure of impulsivity after exposure to cocaine. The cocaine

manipulation increased impulsivity only in rats with an initially low level of baseline impulsivity. Similarly, in the present research, two groups of rats with different levels of baseline impulsivity were tested on a measure of impulsivity after exposure to stress. The stress manipulation increased impulsivity only in the low baseline impulsivity group (Fischers). It should be noted that impulsive action was measured in the present experiment, while impulsive choice was measured in the experiments by Anker et al. (2009) and Barbelivien et al. (2007)--two related but dissociable constructs. It would be interesting to determine whether the same patterns would emerge when examining effects of stress on impulsive choice, and effects of caffeine, amphetamine, and cocaine on impulsive action.

**Finding #3: Attention was correlated with impulsive action.**

Impulsivity and attentional deficits are each components of ADHD, (DSM-IV-TR, 2000), and it has been suggested that impulsivity and attention are closely related constructs (Barkley, 1997; de Wit, 2009). Because Lewis rats are more impulsive than Fischer rats, it was hypothesized that Lewis rats would have decreased attention compared to Fischer rats. Surprisingly, Lewis and Fischer rats did not differ significantly on measures of attention at baseline.

*Attention and Impulsivity*

de Wit (2009) proposed that lapses of attention may represent a separate measure of impulsive tendencies that is dissociable from other measures. In the

present research, the omissions parameter was conceptualized as reflecting lapses in attention, or inattention. While Lewis and Fischer rats differed significantly in impulsive action, they did not differ in inattention. At baseline, measures of impulsive action were correlated with measures of attention; however, the correlations occurred in the opposite direction from that which was reflected in Hypothesis 1D. In Lewis rats, premature responses were negatively correlated with omissions. That is, as lapses in attention increased in Lewis rats, impulsive action decreased. A positive correlation between omissions and premature responses would have provided support for the relationship between impulsivity and inattention reflected in Hypothesis 1D. The negative correlation that occurred between impulsivity and inattention in Lewis rats suggests that inattention and impulsivity are dissociable constructs, consistent with de Wit's (2009) suggestion.

Further, the negative correlation between inattention and impulsivity that occurred in Lewis rats suggests that the relationship between inattention and impulsivity is complex: increases in inattention do not necessarily translate into increases in impulsivity. However, positive correlations occurred between correct responses and impulsive action. The positive correlation between correct responses and impulsive action suggests that impulsivity is associated with increased attention, possibly indicating hyperarousal or a prepotency to respond.

The fact that Lewis rats had better attention than Fischer rats indicates that they would not be good candidates for an animal model of ADHD. While Lewis rats are more impulsive, they do not appear to have attentional deficits.

**Finding #4: Stress decreased attention in both rat strains, with the greatest effect in the Lewis rats.** Stress both increased omissions and decreased correct responses, the two parameters used to measure attention in the present research. This finding is new and was unexpected. The present results indicate that stress is detrimental to attention. It is possible that the emotional state of stress requires allocation of attentional resources that detract from overall attentional performance.

### *Corticotrophin Releasing Factor and Attention*

The finding that stress decreased attention is inconsistent with a report of corticotrophin releasing factor (CRF) enhancing attention in Wistar and Lister hooded rats on the 5-CSRTT (Ohmura et al., 2009). Ohmura et al. (2009) administered CRF intracerebroventricularly and assessed Lister hooded and Wistar rats' performance on the 5-CSRTT. Attention was improved by CRF administration in both rat strains, regardless of their baseline attentional performance, although impulsivity was not affected. Effects of CRF to enhance attention are inconsistent with the present results, in which stress decreased attention in both rat strains.

There are two possible reasons for the inconsistency between the present research and the work of Ohmura et al. (2009). First, it is possible that administration of CRF is not comparable to the experience of stress induction. Psychological stress induction may require allocation of attentional resources to the affective state that detracts from attentional performance on the 5-CSRTT.

CRF administration may not require allocation of the same attentional resources. Second, it is possible that the Lister hooded, Wistar, Lewis, and Fischer rat strains' attentional performances are differentially affected by stress and its neurobiological substrates: Lister hooded rats and Wistar rats' attentional performance is enhanced by CRF, while stress is detrimental to attentional performance in Lewis and Fischer rats. Experiments in which stress was induced using the same methods as those used in the present research would be needed to address these possibilities.

### *Experiment 1 Limitations*

Experiment 1 had some limitations that may have affected the results. First, stressors in Experiment 1 were not presented in a counterbalanced fashion, which does not allow for the ruling out of order effects. Instead, all stress-group rats received immobilization restraint stress on the first day, all stress-group rats received predator stress on the second day, and all stress-group rats received combined immobilization restraint stress and predator stress on the third day. All rats received the same stressor on the same day in Experiment 1, rather than some rats receiving different stressors on the same day, in case the stressors did not induce equal levels of stress. In fact, because effects of stress on impulsive action varied slightly across stress days, it could be argued that the stressors may not have induced equal levels of stress. It should be noted, however, that because stressors were not counterbalanced in Experiment 1, it is not possible to rule out order effects of the stressors, including

carryover effects. Second, animals were separated during 5-CSRTT testing, which may have caused stress in the non-stress groups. Separation of the rats during testing was necessary, however, because the 5-CSRTT procedure can only accommodate one rat at a time. Steps were taken to minimize any effects of separation stress by testing cagemates at the same time, so that time spent apart was not prolonged by the absence of one cagemate, and no rat spent time in the homecage alone. Third, loud noises that occurred outside the testing room on Stress Day 1 may have stressed the non-stress group rats. However, the loud noises were outside the experimenter's control, and occurred only on the first stress day. Lastly, only impulsive action was measured in the present experiment. Future research should examine effects of stress on impulsive choice in Lewis and Fischer rats.

*Stress, Impulsive Action, and Nicotine Behavioral Sensitization.* This research examined two possible psychological mechanisms underlying the stress and cigarette smoking relationship in impulsive and non-impulsive individuals: an effect of stress to increase impulsivity, and an effect of stress to increase reinforcing actions of nicotine. Experiment 1 determined the effect of stress on impulsive action in impulsive and non-impulsive rats. The purpose of Experiment 2 was to determine the effect of stress on reinforcing actions of nicotine in impulsive and non-impulsive rats.

# EXPERIMENT 2: The Effects of Stress and Impulsivity on Behavioral Sensitization to Nicotine

## Methods

### *Overview*

As depicted in the timeline in Appendix A, Experiment 2 determined the effect of acute stress and previously existing impulsive action on behavioral sensitization to nicotine in Lewis and Fischer rats. The same rats that were stressed in Experiment 1 also were stressed in Experiment 2, and all rats received daily injections of nicotine. The effect of stress on nicotine-induced locomotor activity was assessed each day immediately after injections. Additionally, impulsive action was measured throughout Experiment 2 to examine effects of repeated nicotine administration on impulsive action.

### *Purpose*

Experiment 2 examined the effects of acute psychological stress and previously existing impulsive action on behavioral sensitization to 0.5 mg/kg nicotine. Effects of nicotine sensitization and stress on impulsive action also were determined.

### *Hypotheses*

In Experiment 2, hypotheses 2A and 2B address the effect of stress on corticosterone levels. Hypotheses 3A-3E address the effect of impulsivity (either based on rat strain or impulsive action) and stress on behavioral sensitization to

nicotine. Hypotheses 4A-4D address the effect of repeated nicotine administration on impulsive action and attention.

*Stress:*

**Hypothesis 2A:** Stress will increase blood corticosterone levels in Lewis and Fischer rats.

The experience of psychological stress sets in motion a cascade of physiological events, the end result of which is a surge in circulating cortisol levels. In rats, the hormone corticosterone is equivalent to human cortisol hormone. Corticosterone levels are detectable in rat blood and are often measured in experimental investigations to assess whether a rat was stressed (Acri, 1994; Belz, Kennell, Czambel, Rubin, & Rhodes, 2003; Brown & Grunberg, 1995; Faraday, 2002; Faraday, Blakeman, & Grunberg, 2005; Hayley et al., 2001; Kant et al., 1987; Raygada, et al., 1992). In stressed rats, circulating levels of corticosterone are elevated. Therefore, it was hypothesized that corticosterone levels would be elevated in stressed Lewis and Fischer rats.

**Hypothesis 2B:** Corticosterone will be elevated in stressed and non-stressed Fischer rats as compared with stressed and non-stressed Lewis rats.

Lewis rats are hyporesponsive to stress as compared with Fischer rats, as reflected by lower corticosterone and adrenocorticotrophin (ACTH) levels both in response to a stressor (Chaouloff et al., 1995; Dhabar et al., 1993) and at rest (Dhabar et al., 1993). Fischer rats have augmented biochemical responses to stress compared with Lewis rats, as well as at rest.

*Behavioral Sensitization to Nicotine:*

**Hypothesis 3A:** Behavioral sensitization to nicotine will be greater in Lewis rats than Fischer rats.

Rationale: Lewis rats are more sensitive to nicotine and demonstrate increased incentive motivation to nicotine compared to Fischer rats (Brower et al., 2002; Pescatore et al., 2005; Philibin et al., 2005; Suzuki et al., 1999). Behavioral sensitization reflects increased incentive motivation (Robinson & Berridge, 1993, 2000, 2003; 2008), a mechanism of which is increased DA neurotransmission (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987). DA neurotransmission is greater in Lewis than Fischer rats (Camp et al., 1994; Flores, et al., 1998; Kosten & Ambrosi, 2002; Strecker et al., 1995). Additionally, Lewis rats have greater incentive motivation for nicotine than Fischer rats (Horan et al., 1997; Pescatore et al., 2005; Philibin et al., 2005). Therefore, it was hypothesized that behavioral sensitization to nicotine will be greater in Lewis rats than in Fischer rats, as was found in a previously conducted experiment (Hamilton et al., *under review*).

**Hypothesis 3B:** Psychological stress will increase behavioral sensitization to nicotine in Lewis and Fischer rats.

Rationale: Increased dopamine neurotransmission is a mechanism underlying behavioral sensitization (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987) and stress increases dopamine release (Abercrombie et al., 1989; Finlay et al., 1995; Oswald et al., 2005;

Pruessner et al., 2004). Therefore, it was hypothesized that effects of stress and nicotine behavioral sensitization will combine to increase behavioral sensitization to nicotine in Fischer and Lewis rats.

**Hypothesis 3C:** The effect of stress to increase nicotine behavioral sensitization will be greater in Lewis rats than in Fischer rats.

Rationale: In rodents, stress increases drug seeking, drug craving, and self-administration of substances, including nicotine (Buczek et al., 1999; Le et al., 1998; Shaham et al., 1993; Soloff et al., 2000). Lewis rats are more sensitive to nicotine (Horan et al., 1997; Pescatore et al., 2005; Philibin et al., 2005) and have greater DA neurotransmission (Camp et al., 1994; Kosten & Ambrosi, 2002; Flores et al., 1998; Strecker et al., 1995) than Fischer rats. DA neurotransmission is implicated in behavioral sensitization (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987) and is increased by stress (Abercrombie et al., 1989). Therefore, it was hypothesized that the effect of stress to increase behavioral sensitization to nicotine will be greater in Lewis than in Fischer rats.

**Hypothesis 3D:** Impulsive action before drug administration will statistically predict behavioral sensitization to nicotine.

Rationale: Increased DA neurotransmission is a mechanism of both impulsive action (van Gaalen et al., 2006) and behavioral sensitization (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987). Rats with high levels of impulsive action have increased dopamine neurotransmission, which will be further increased by behavioral sensitization.

Therefore, it was hypothesized that impulsive action before drug administration will statistically predict behavioral sensitization to nicotine.

Additionally, impulsivity is associated with cigarette smoking in humans (Mitchell, 1999; Bickel, 1999) and nicotine self-administration in rats (Diergaarde et al., 2008). Specifically, impulsive action was associated with an enhanced motivation to initiate and maintain nicotine self-administration (Diergaarde et al., 2008). Enhanced nicotine reinforcement in impulsive individuals may be a mechanism underlying the reported findings. For this reason, it was hypothesized that impulsivity will predict behavioral sensitization to nicotine.

**Hypothesis 3E:** Impulsive action before drug administration will statistically predict nicotine behavioral sensitization to a greater degree in Lewis rats than in Fischer rats.

Rationale: Increased DA neurotransmission is a mechanism of both impulsive action (van Gaalen et al., 2006) and behavioral sensitization (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987). Lewis rats have higher levels of DA neurotransmission (Camp et al., 1994; Flores, et al., 1998; Kosten & Ambrosi, 2002; Strecker et al., 1995) and have more impulsive action (Kearns et al., 2006) than Fischer rats. Therefore, it is hypothesized that impulsive action before drug administration will statistically predict behavioral sensitization to a greater degree in Lewis rats than in Fischer rats.

Additionally, impulsivity is associated with cigarette smoking in humans (Mitchell, 1999; Bickel, 1999) and nicotine self-administration in rats (Diergaarde

et al., 2008), with impulsive action associated with an enhanced motivation to initiate and maintain nicotine self-administration (Diergaarde et al., 2008). Enhanced nicotine reinforcement in impulsive individuals may be a mechanism underlying the reported findings. Lewis rats are more impulsive than Fischer rats on a measure of impulsive choice (Anderson & Woolverton, 2005), and Lewis rats have a greater nicotine preference and intake than Fischer rats (Brower, Fu, Matta, & Sharp, 2002; Horan, Smith, Gardner, Lepore, & Ashby, 1997; Philibin, Vann, Varvel, Covington, Rosencrans, James, & Robinson, 2005). Because impulsivity is associated with nicotine self-administration, and Lewis rats have greater nicotine intake and preference and are more impulsive compared to Fischer rats, it is hypothesized that the relationship between impulsivity and nicotine behavioral sensitization will be stronger in Lewis rats.

*Impulsive action and attention:*

**Hypothesis 4A:** Nicotine behavioral sensitization will increase impulsive action in Lewis and Fischer rats.

Rationale: Blondel, Sanger, and Moser (2003) reported that acute nicotine administration increased impulsivity in the 5-CSRTT in Sprague-Dawley rats. Blondel et al. (2003) administered via intraperitoneal injections two doses of nicotine (0.1 mg/kg nicotine and 0.3 mg/kg nicotine), repeatedly for five days in Sprague Dawley rats. In the present experiment, 0.5 mg/kg nicotine was administered via subcutaneous injections to rats of the Lewis and Fischer rat strains. The 0.5 mg/kg nicotine dose was used because it is the optimal dose to

observe nicotine behavioral sensitization (Di Franza & Wellman, 2007). It was expected that the results of the Blondel group will be replicated in the present experiment. Increased DA neurotransmission is a mechanism of both impulsive action (van Gaalen et al., 2006) and behavioral sensitization (Hamamura et al., 1991; Kalivas & Stewart, 1991; Robinson & Berridge, 1993; Wise, 1987). Therefore, it was predicted that behavioral sensitization to nicotine will increase impulsivity over time in Lewis and Fischer rats.

**Hypothesis 4B:** Nicotine behavioral sensitization will increase impulsive action in stressed rats more than in non-stressed rats.

Rationale: It was predicted that behavioral sensitization to nicotine will increase impulsive action, both of which are mediated by increased dopamine neurotransmission (van Gaalen et al., 2006). Stress increases dopamine release (Abercrombie et al., 1989). Therefore, it was hypothesized that effects of nicotine behavioral sensitization to increase impulsive action will be greater in stressed rats than in non-stressed rats.

**Hypothesis 4C:** The effect of nicotine behavioral sensitization to increase impulsive action will be greatest in Lewis stressed rats.

Rationale: It was predicted that behavioral sensitization to nicotine will be greater in Lewis rats than in Fischer rats. Additionally, it is predicted that behavioral sensitization to nicotine will increase impulsive action, both of which are mediated by increased dopamine neurotransmission (van Gaalen et al., 2006). Therefore, it was predicted that effects of stress, rat strain, and nicotine

behavioral sensitization will combine so that nicotine behavioral sensitization will cause the greatest increase in impulsive action in stressed Lewis rats.

**Hypothesis 4D:** Nicotine will increase attention in the 5-CSRTT in Lewis and Fischer rats.

Rationale: In previous research, nicotine increased sustained attention or vigilance (Blondel et al., 2000) and enhanced selective attention (ability to ignore irrelevant stimuli) (Hahn et al., 2002) in the 5-CSRTT. Therefore, it was predicted that nicotine will enhance attention in the present experiment in Lewis and Fischer rats.

### *Experimental Design*

The same rats used in Experiment 1 were used in Experiments 2a and 2b.

### *Power analysis*

Calculations based on data from the previously conducted study, reported above, indicate that the observed effect size for the main effect of rat strain was 1.48 and the observed effect size for the main effect of stress was 1.45. Effect size calculations were based on data from the rats that received nicotine. A cell size of 6 rats would be sufficient to detect main effects of stress and rat strain on behavioral sensitization at 80% power with an alpha level of $p < 0.05$. Calculations based on data from the previously conducted study indicate that the observed effect size for the stress x rat strain two-way interaction was 0.46. A cell size of 38 rats would be needed to detect an effect size of 80% power with an alpha level of $p < 0.05$. Because of the length of time required for training on the 5-CSRTT, a cell size of 38 rats (N=142) would not be feasible for the present research. For this reason, a cell size that is sufficient to detect main effects of stress and rat strain was used.

A cell size of 6 rats would be sufficient to determine main effects of stress and rat strain on behavioral sensitization to nicotine. However, there was a possibility that attrition would occur during repeated nicotine administration. To account for a decrease in power that could result from attrition, a cell size of 8 rats was used in the present research.

With regard to the within-subject factors of nicotine behavioral sensitization and impulsive action, a cell size of 8 rats per group had 80% power to detect a change in the difference between groups of 0.17 standard deviations per day (totaling 1.2 standard deviations over the 7 day period) based on a general linear model with group as a between-subjects factor and time as a continuous within-subject factor (Diggle, Heagerty, Liang, & Zeger, 2002).

Additionally, power was simulated by generating and analyzing 500 data sets with computer software for a 2 x 2 repeated measures design with two between-subject factors (stress and rat strain) and one within-subject factor (time) using SAS version 9.0. The within-subject correlation for the simulation was set at 50%, and alpha was set at 0.05. For effect size, it was assumed that over the seven-day period, the difference between rat strains would increase by 1.25 standard deviations total (0.18 standard deviations per day) and that the difference between the stress and no stress conditions would also increase by 1.25 standard deviations total (0.18 standard deviations per day). Additionally, an interaction effect size of 0.75 standard deviations over the seven-day period was assumed, indicating that the difference in increase over seven days between Lewis and Fischer rats would be 0.75 standard deviations larger in the stressed rats.

According to the simulation that used computer-generated data, a sample size of 8 per group would have 87% power to detect significant stress x time and rat strain x time interactions, but only 10% power for the stress x rat strain x time interaction. Similarly, if a 2-way ANOVA were conducted at drug day 7, there

would be 93% power to detect main effects of stress and rat strain but only 11% for the stress x rat strain x time interaction. For the within-subject factor, a sample size of 6 per group would have 80% power to detect significant stress x time and rat strain x time interactions, but only 10% power for the stress x rat strain x time interaction. Similarly, for the between-subjects factor, if a 2-way ANOVA were conducted at drug day 7, there would be 84% power to detect main effects of stress and rat strain but only 10% for the stress x rat strain interaction. For these reasons, the present experiment was powered to detect effects of stress x time and rat strain x time.

The simulations suggested that 6 rats per group would be sufficient to detect rat strain x time and stress x time interactions. However, to detect an interaction of rat strain x stress x time, approximately 100 rats per group would be needed, for a total N = 400. For this reason, the experiment was powered to detect main effects of stress and rat strain, and interactions of stress x time and rat strain x time. However, as discussed above, based on two studies of impulsive action in which approximately 90-94% of rats met the training criterion (Talpos et al., 2006; Kearns et al., 2006), there was a possibility that not all rats would learn the impulsivity task (personal communications, 2009). Additionally, attrition may have occurred over the 10 to 12 weeks required for 5-CSRTT training and during behavioral sensitization to nicotine.

A cell size of 6 rats would be sufficient to determine main effects of stress and rat strain. However, there was a possibility that attrition could occur over the 10 to 12 weeks required for 5-CSRTT training. To account for a decrease in

power that could result from attrition or failure to meet a training criterion, a cell size of 8 rats was used in the present research.

### *Subjects and Housing*

Subjects were the same 16 adult male Lewis rats and 16 adult male Fischer rats used in Experiment 1 (Charles River Laboratories). Housing was the same in Experiment 2 as it was in Experiment 1. Briefly, rats were pair housed within rat strain and maintained on a reverse light cycle at 85-90% free-feeding body weight. As in Experiment 1, animals were maintained at 85% to 90 % free-feeding body weight to motivate performance on the 5-CSRTT in Experiment 2B, and to maintain health. The procedure for maintaining rats' body weight at 85% to 90% of their free-feeding body weight was identical to that used in Experiment 1, as described above.

### *Independent Variables*

There were three independent variables (IVs) in the present experiment: Rat Strain, Stress, and Time. There were two levels of the stress (stress and non-stress) and rat strain (Fischer and Lewis) between-subjects variables. Stress was induced in a counterbalanced fashion using the same stress procedures as were used in Experiment 1, as depicted in the Stress Timeline below (Table 5). Additionally, time was a within-subject variable.

*Dependent Variables*

The same dependent variables used in Experiment 1 also were measured in Experiment 2. The main dependent variable in Experiment 2a was behavioral sensitization to nicotine, which is reflected by an increase in horizontal locomotor activity over time during the administration of the drug. Therefore, while locomotor activity was used in Experiment 1 to reflect general movement, the measurement reflected reactivity to nicotine in Experiment 2a. An additional dependent variable measured in Experiment 2 was blood corticosterone levels. Measurement of corticosterone levels provided a manipulation check, verifying that stress occurred in rats assigned to the stress group, and allowed for the comparison of stress effects in the Lewis and Fischer rat strains. Additionally, body weight was measured. In Experiment 1, body weight was measured to verify that rats were maintained at 85% - 90% body weight. While body weight measurements were used for this purpose during Experiment 2, the measurements also were used daily to adjust the nicotine solution, so that each rat was given a 0.5 mg/kg nicotine dosage. Impulsive action measurements collected during Experiment 1 (prior to drug administration) were used to account for the effect of impulsive action on nicotine sensitization during stress. Impulsive action measurements collected in Experiment 2b were used to determine whether daily nicotine administration affects impulsive action. The main dependent variable in Experiment 2b was impulsive action, as measured by the 5-CSRTT. Body weight also was measured daily during Experiment 2b. All DVs were collected in the manner described above.

## *Procedure*

### *Serum Corticosterone*

As described in the introduction, psychological stress sets in motion a cascade of physiological events, the end result of which is a surge in circulating cortisol levels. In rats, the hormone corticosterone is equivalent to human cortisol hormone. Corticosterone levels are detectable in rat blood and are often measured in experimental investigations to assess whether a rat was stressed (Acri, 1994; Belz, Kennell, Czambel, Rubin, & Rhodes, 2003; Berger, 2009; Brown & Grunberg, 1995; Faraday, 2000; Faraday, 2002; Faraday, Blakeman, & Grunberg, 2005; Hayley et al., 2001; Kant et al., 1987; Perry, 2009; Raygada, et al., 1992). In the present research, corticosterone levels were measured at the conclusion of the experiments to verify that the Lewis and Fischer rats that received stress induction were stressed. Additionally, corticosterone levels were measured to compare stress responses between the Fischer and Lewis rat strains.

After the completion of the experiments, subjects were anesthetized by carbon dioxide inhalation and decapitated with a rat guillotine, following procedures approved by LAM, in order to collect and centrifuge blood for serum corticosterone assay. Following the procedures of Berger (2009) and Perry (2009), serum corticosterone was assayed by an ImmuChem Double-Antibody radioimmunoassay (RIA) kit using $^{125}$ I-labeled corticosterone (ICN Biomedicals, Costa Mesa, CA). In the assay, a limited quantity of specific antibody reacts with a fixed amount of $^{125}$ I-labeled corticosterone. The corticosterone concentration

recorded. Injections were always administered in the same room, the room in which locomotor activity testing was conducted. Rats were tested on the locomotor activity chambers in two counterbalanced cohorts, with each condition equally represented in each cohort.

*Baseline Phase*

During the Baseline Phase, all rats received dorsal SC injections of 1 ml saline via 5/8" 26 gauge needles attached to 3 mL syringes in the locomotor activity room prior to locomotor activity measurement. This acclimated the rats to receiving injections, and allowed for the assessment of locomotor activity in response to saline for all rats.

*Stress Phase*

After three days of saline administration, stress was induced daily, immediately prior to nicotine injections and locomotor activity measurements. Stress was induced in a counterbalanced fashion, as depicted in the stress induction timeline in Table 5, to ensure that any variance due to either the order in which stressors were presented or the type of stressor given on a particular day was evenly distributed across groups. Stress was induced by a varied combination of immobilization restraint stress, predator stress, and exposure to unpredictable stimuli. On the day rats were euthanized by decapitation in a rat guillotine, after being anesthetized by $CO_2$, stress was induced in stress-group rats immediately prior to euthanization using the same procedure for all rats

(combined restrainer and predator stress with coin shake) to minimize variance in cortiocosterone levels that may have occurred from using different types of stressors.

Table 5. Stress Induction Timeline: Experiment 2

| Day | Procedure A1 Cohorts 1 and 2 | Procedure B1 Cohorts 3 and 4 | Procedure A2 Cohorts 5 and 6 | Procedure B2 Cohorts 7 and 8 |
|---|---|---|---|---|
| 1 | Restraint stress + whistle | Predator Stress + whistle | Predator stress + cage shake | Restraint stress + restrainer shake |
| 2 | Restraint stress + restrainer shake | Restraint stress | Restraint Stress + alarm clock | Predator stress + alarm clock |
| 3 | Predator stress + Alarm clock | Restraint Stress + Alarm Clock | Restraint + coin shake | Predator stress + coin shake |
| 4 | Restraint stress + flashing lights | Predator stress + flashing lights | Predator stress + cage shake | Cage Shake + coin shake |
| 5 | Whistle + alarm clock | Predator stress + whistle + alarm clock | Restraint stress + restrainer shake | Predator stress + restraint stress |
| 6 | Predator stress + restraint stress | Restraint stress + restrainer shake | Whistle + alarm clock | Predator stress + whistle + alarm clock |
| 7 | Predator stress + cage shake | Cage Shake + coin shake | Predator stress + flashing lights | Restraint stress + flashing lights |
| 8 | Restraint + coin shake | Predator stress + coin shake | Predator stress + Alarm clock | Restraint Stress + Alarm Clock |
| 9 | Predator Stress + alarm clock | Restraint stress + alarm clock | Restraint stress + restrainer shake | Restraint stress |
| 10 | Predator stress + cage shake | Restraint stress + cage shake | Restraint stress + whistle | Predator Stress + whistle |
| 11 | Predator Stress + coin shake | Restraint Stress + Coin Shake | Predator Stress and Flashing Lights | Restraint Stress + Flashing Lights |
| 12 | Predator Stress + Restraint Stress + coin shake | Predator Stress + Restraint Stress + coin shake | Predator Stress + Restraint Stress + coin shake | Predator Stress + Restraint Stress + coin shake |

*Euthanasia*

Between 9:00 a.m. to 12:30 p.m. on the last day of Experiment 2, rats were euthanized following procedures approved by the Uniformed Services University of the Health Sciences Institutional Animal Care and Use Committee (IACUC). Rats were anesthetized with $CO_2$ and decapitated with a rat guillotine. Immediately prior to euthanization, all stress-group rats were stressed by exposure to combined restrainer and predator stress with coin shake. Brains were collected for future research and flash frozen in a container of methylbutane surrounded by dry ice. Trunk blood was collected and centrifuged for serum corticosterone assay (described above).

*Experiment 2 Data Analytic Strategy*

*Locomotor activity.* Locomotor data were analyzed with repeated-measures analysis of variance (ANOVA) with rat strain and stress group as the between-subjects factors, and time as the within-subject factor. Analysis of time as the within-subject factor suggested whether behavioral sensitization to nicotine occurred. However this determination could not be made unequivocally as a placebo condition was not included in the experiment to account for non-specific effects of time on locomotor activity. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs. If the assumption of sphericity was violated, as revealed by a Mauchly's test, then a Greenhouse-Geisser correction

was used. All tests were two-tailed and an $\alpha$ level of 0.05 was used to determine statistical significance.

*Locomotor activity: Baseline Day and Saline Days.* Univariate ANOVAs were conducted individually on each day of locomotor activity on all days before nicotine was administered (Baseline Day and Saline Days), with stress and rat strain as between-subjects factors to determine whether there were any significant differences between groups prior to stress and nicotine administration. Additionally, a repeated-measures ANOVA was conducted on locomotor activity on all days prior to nicotine administration.

*Locomotor activity: Nicotine-administration days.* Analyses revealed significant differences between groups prior to nicotine administration. For this reason, locomotor activity on saline day 3 was used as a covariate, because saline day 3 was temporally the closest to nicotine administration. For all nicotine-administration days, locomotor activity data were analyzed using repeated-measures ANCOVA with stress and rat strain as between-subjects factors, day as the within-subject factor, and saline day 3 activity as a covariate. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANCOVAs and additional repeated-measures ANCOVAs with saline day 3 activity as the covariate.

*Locomotor activity: Post-Nicotine Day 3.* Locomotor activity data were analyzed using a univariate ANCOVA, with stress and rat strain as between-subject factors and saline day 3 activity as a covariate.

that is unlabeled in samples increases as a function of the decreasing percentages of bound radioisotope-labeled corticosterone (Berger, 2009; Perry, 2009; Long, 2010). Then, the addition of a second antibody causes the precipitation of antibody bound to antigen. At the conclusion of the procedure, the quantity of endogenous corticosterone in samples was determined by measuring in a gamma counter the radioactivity of the precipitate with known standards from the same assay. The disintegrations per minute (DPM), a measure of radioactivity, was converted into concentrations. All samples and standards were processed in duplicate pairs. The sensitivity of the assay is 8 ng/ml (Faraday, 2000) and the coefficient of variation is <7% (Berger, 2009; Perry, 2009).

*Impulsivity Assessment*

All rats were tested for impulsive action in the 5-CSRTT prior to the initiation of stress induction and drug administration (Experiment 1). Scores on the behavioral measure of impulsivity were used to determine whether impulsive action predicts nicotine behavioral sensitization on any days of drug administration. During Experiment 2, impulsive action was assessed every other day to determine whether it changes before, during, and after nicotine behavioral sensitization, and to determine whether any changes that occur differ between the two rat strains. The number of 5-CSRTT testing chambers was a limiting factor in the present research. To account for this, rats were divided into two cohorts and each cohort was tested in the 5-CSRTT on alternating days after

locomotor activity testing. Within each testing day in Experiment 2, the order in which rats were tested was counterbalanced to account for order effects.

*Attention Assessment*

Procedures for assessing attention in Experiment 2 were identical to procedures used to assess attention in Experiment 1.

*Behavioral Sensitization*

Data collection for Experiment 2 took 14 days. Behavioral sensitization was measured using an Omnitech/Accuscan Electronics Digiscan infrared photocell activity system located in a dedicated room. Rats were tested on the measures of impulsivity over the course of one week prior to the initiation of the experiment (Experiment 1). Rats were acclimated to the locomotion chambers for two days, and received SC saline injections on the dorsal side immediately prior to locomotor activity measurements for three days before nicotine injections began. Beginning on day 96, all rats received daily SC injections of 0.5 mg/kg nicotine for 8 days. Rats received daily nicotine injections for 8 days because nicotine sensitization is generally maximal within 5-7 days of drug administration (Kempsill & Pratt, 2000; DiFranza & Wellman, 2007). Stress induction occurred immediately prior to nicotine injections for all stress-group rats. Stress was induced by a varied combination of immobilization restraint stress, predator stress, and unpredictable stimuli. On all drug administration days, rats were placed immediately into the activity chambers after injections, and data were

*Regression analyses.* A simple linear regression was used to examine the proportion of variance in behavioral sensitization that was accounted for by the amount of impulsive action in rats that was measured in Experiment 1 on each nicotine administration day. Additionally, interaction terms for baseline premature responses x rat strain, baseline premature responses x stress, rat strain x stress, and baseline premature responses x rat strain x stress were calculated. These terms were the independent variables while horizontal activity for each variable was a dependent variable. Linear regression analyses were conducted for each day to determine whether the interaction terms predicted locomotor activity. All tests were two-tailed with an $\alpha$ level of $p = 0.05$.

*Impulsive action and attention.* 5-CSRTT data for impulsivity and attention were analyzed using mixed model analyses. The mixed model analyses were used to analyze both cohorts together. During the saline administration phase, impulsivity and attention were analyzed using a two-way ANOVA.

During the nicotine administration days, rats continued to be tested on the 5-CSRTT in two cohorts on alternating days. However, an equipment failure on Drug Day 4 caused impulsive action and attention data on that day and all remaining days to be lost. Therefore, the available 5-CSRTT data from the nicotine administration days were Drug Days 1 and 3 for Cohort A, and Drug Day 2 for Cohort B. To accomodate the missing data points, mixed model analyses were performed. Mixed model analyses use a regression to predict the values of missing datapoints, and therefore allowed Cohorts A and B to be included in the same analysis.

*Corticosterone.* Corticosterone data were analyzed using a univariate ANOVA with stress and rat strain as the between-subjects variables. All tests were two-tailed with an $\alpha$ level of $p = 0.05$.

*Sample size.* An N of 32 rats was used in the present experiment, with 8 rats per cell. This cell size was based on the power analysis described above.

*Missing data points.* For some analyses, degrees of freedom were not consistent with N=32. These discrepancies resulted from two factors. First, rat 401 died during the nicotine administration phase of Experiment 2, on Drug Day 3 after the locomotor activity measurement. Locomotor Activity data were lost for four rats on Drug Day 4, and for four different rats on Drug Day 5.

# RESULTS

## EXPERIMENT 2: The Effects of Stress and Impulsivity on Behavioral Sensitization to Nicotine

The findings for Experiment 2 are reported below. Presentation of the experimental findings organized by dependent variable follows, in the order of locomotor activity results, impulsive action results, attention results, and corticosterone results, respectively. The findings are illustrated in graphs interspersed throughout the results section text, and the statistics supporting each finding are presented in statistics tables in Appendix D.

### Results

*Corticosterone.* Stressed rats had higher corticosterone levels than non-stressed rats [$F(1,27) = 21.171$, $p < 0.001$] and Fischer rats had higher corticosterone levels than Lewis rats [$F(1,27) = 38.647$ $p < 0.001$]. The corticosterone results are displayed in Figure 14, and corresponding statistics are presented in Table 10A.

*Summary: Corticosterone.* Corticosterone levels were elevated in stressed rats compared to non-stressed rats, and in Fischer rats compared to Lewis rats.

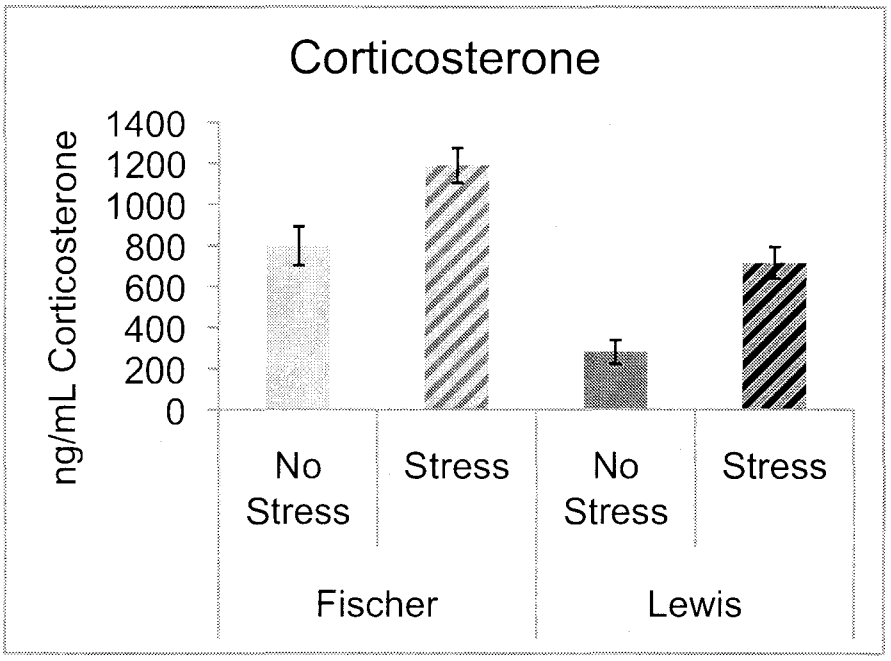



Figure 14. Blood corticosterone levels (ng/mL) in stressed and non-stressed Lewis and Fischer rats.

*Confirmation of Hypotheses.* **The hypothesis that stressed rats would have higher corticosterone levels than non-stressed rats (Hypothesis 2A) was confirmed. The hypothesis that Fischer rats would have higher corticosterone levels than Lewis rats was confirmed also (Hypothesis 2B).**

*Locomotor Activity*

*Baseline Day and Saline Days.* Activity varied among stress and rat strain groups over time [F(3,84) = 3.89, p = 0.012]. Activity varied across days in Lewis rats [F(3,42) = 6.792, p = 0.001] and Fischer rats [(3,42) = 6.131, p = 0.001]. Effects of stress varied across days in Fischer rats [F(3,42) = 4.124, p = 0.012], and stress decreased locomotor activity in the Fischer rat strain [F(1,14) = 8.629,

$p = 0.011$]. Additionally, in the stress group activity varied across days [$F(3,42) = 9.367$, $p < 0.001$], and varied across days differently in Lewis and Fischer rats [$F(3,42) = 2.986$, $p < 0.05$]. In the non-stressed rats, activity varied across days [$F(3,42) = 3.329$, $p < 0.05$] and differed between Lewis and Fischer rats [$F(1,14) = 11.711$, $p = 0.004$] (see Figure 15 and Tables 11A, 11B, and 11C in Appendix D).

On Baseline Day, locomotor activity did not differ among stress and rat strain groups (Table 12A, Appendix D). On Saline Day 1, the greatest amount of locomotor activity occurred in the Fischer non-stress group rats and the least amount of activity occurred in Fischer stress group rats and the Lewis non-stress group rats [$F(1,28) = 10.13$, $p < 0.01$] (Table 13A, Appendix D). Although differences occurred among stress groups, rats were not stressed during the Baseline and Saline Administration Phase. On Saline Day 2, Fischer rats had more locomotor activity than Lewis rats [$F(1,28) = 7.773$, $p < 0.01$] (Table 14A, Appendix D). On Saline Day 3, Fischer rats had more locomotor activity than Lewis rats [$F(1,28) = 4.206$, $p = 0.05$], non-stress group rats had more locomotor activity than stress group rats [$F(1,28) = 11.924$, $p < 0.01$], and Fischer non-stress group rats had more locomotor activity than all other rats [$F(1,28) = 12.870$, $p < 0.01$] (see Figure 15 and Tables 15A, 15B, and 15C; Appendix D).

Non-stress group Fischer rats had more locomotor activity than stress-group Fischer rats on Saline Day 1 [$F(1,14) = 7.775$, $p = 0.015$] and Saline Day 3 [$F(1,14) = 19.959$, $p = 0.001$]. The present results are presented in Tables 13C and 15C of Appendix D. Additionally, Fischer non-stressed rats had greater

activity than Lewis non-stressed rats on Saline Day 1 [$F(1,14) = 10.705$, $p < 0.01$], Saline Day 2 [$F(1,14) = 7.490$, $p = 0.016$] and Saline Day 3 [$F(1,14) = 17.898$, $p = 0.001$] (see Figure 15 and Tables 13C, 14C, and 15C of Appendix D).

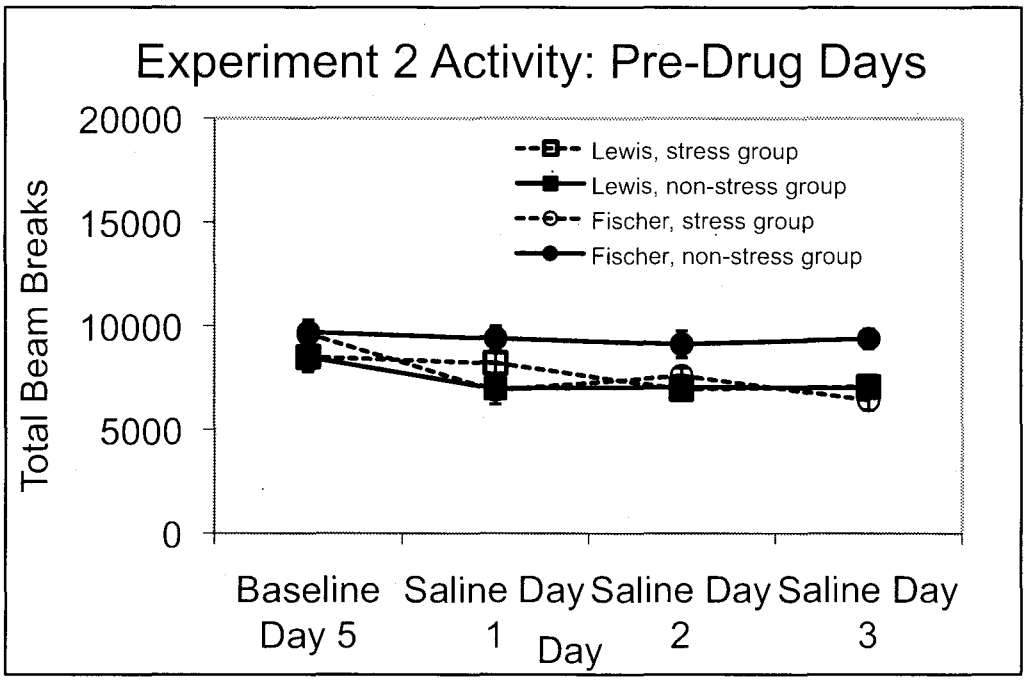



Figure 15. Experiment 2 locomotor activity on Baseline and Saline Administration Days in Lewis and Fischer stress group and non-stress group rats (mean ± S.E.M.).

*Nicotine Administration Days.* Activity varied over time in each stress and rat strain group [$F(3.223, 61.246) = 2.769$, $p < 0.05$] and Lewis rats had more activity overall than Fischer rats [$F(1,19) = 5.066$, $p < 0.05$]. Locomotor activity varied over time in Lewis rats [$F(7,63) = 6.635$, $p < 0.001$] and stressed rats [$F(3.101, 27.905) = 3.852$, $p = 0.019$]. Activity varied in Lewis and Fischer rats

across days [F(3.101, 27.905) = 4.757, p < 0.01] and was greater in Lewis stressed rats than Fischer stressed rats [F(1,9) = 20.826, p = 0.001] (see Figure 16 and Tables 16A-J of Appendix D).

On Drug Day 1, Fischer rats had the most locomotor activity [F(1,27) = 7.873, p < 0.01]. Lewis rats had the most locomotor activity on Drug Day 2 [F(1,27) = 5.217, p < 0.05], Drug Day 3 [F(1,27) = 4.494, p < 0.05], and Drug Day 4 [F(1,22) = 9.950, p < 0.01] (see Tables 17A-H of Appendix D).

Stressed Lewis rats had more activity than stressed Fischer rats on Drug Day 3 [F(1,13) = 20.129, p < 0.01], and Drug Day 4 [F(1,12) = 9.747, p < 0.01], while Fischer stressed rats had more activity than Lewis stressed rats on Drug Day 1 [F(1,13) = 7.631, p = 0.016]. Lewis non-stressed rats had more activity than Fischer non-stressed rats on Drug Day 4 [F (1,9) = 17.028, p < 0.01] (see Figure 16 and Tables 18A-18P and 19A-19P of Appendix D).

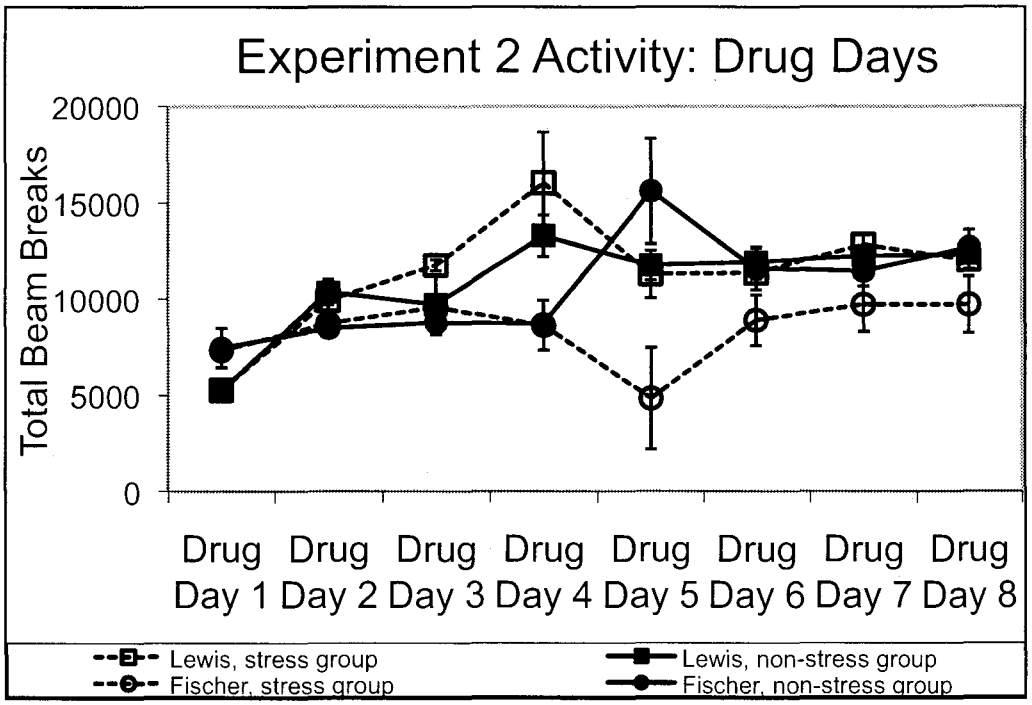


Figure 16. Experiment 2 locomotor activity during nicotine administration in Lewis and Fischer stressed and non-stressed rats (mean ± S.E.M.).

*Post-Nicotine Day 3.* Stressed rats had more activity than non-stressed rats in both rat strains [$F(1,26) = 7.403$, $p = 0.011$] and in Lewis rats [$F(1,13) = 5.382$, $p < 0.05$] (see Figure 17 and Tables 20A, 20B, and 20C).

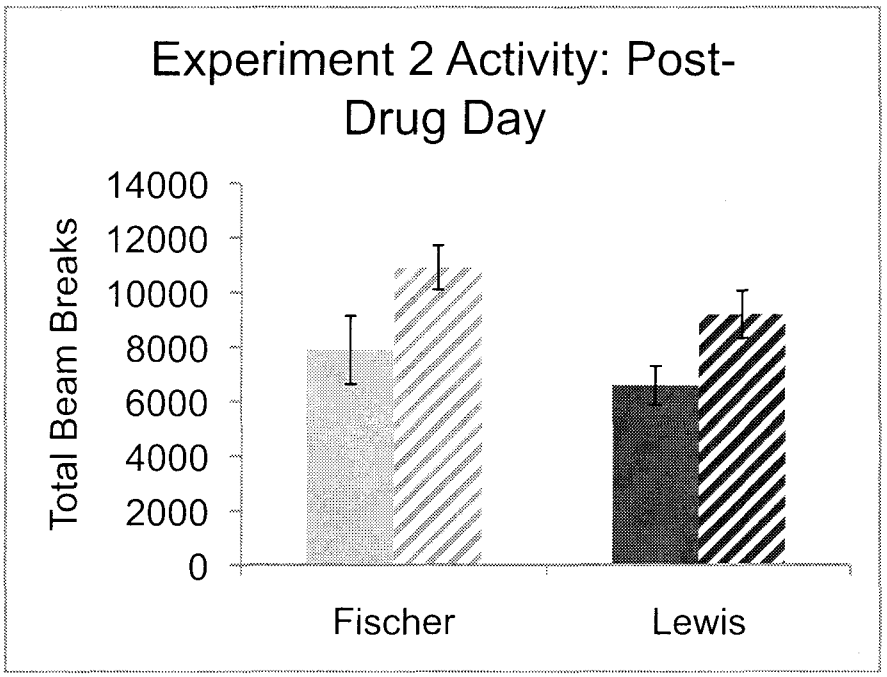



Figure 17. Experiment 2 Post-Drug Locomotor Activity in stressed (striped bars) and non-stressed (solid bars) Lewis and Fischer rats (mean ± S.E.M.).

*Summary: Locomotor Activity.* In Experiment 2, locomotor activity differed between groups during the saline phase. These differences were accounted for statistically in the nicotine administration phase by using Saline Day 3 locomotor activity as a covariate for the locomotor analyses during the nicotine administration and post-drug phases. Nicotine-induced locomotor activity differed among groups across all nicotine administration days. The

patterns of locomotor activity reveal that nicotine behavioral sensitization occurred in all Lewis rats and in non-stressed Fischer rats, but did not occur in stressed Fischer rats. On Post-Drug Day 3, stressed rats had more locomotor activity than did non-stressed rats in both rat strains.

*Regression analyses.* More baseline impulsive action was associated with more nicotine-induced activity on Drug Day 4 [$F(1,26) = 22.464$, $p < 0.001$]. Additionally, the interaction between Baseline Day impulsive action and rat strain predicted locomotor activity on Drug Day 5 [$t=3.411$, $p < 0.01$] and Drug Day 6 [$t=2.662$, $p = 0.013$]. The interaction among baseline impulsive action, rat strain, and stress predicted nicotine-induced locomotor activity on Drug Day 1 [$t=2.282$, $p < 0.05$] (see Tables 23A-I In Appendix D).

*Confirmation of Hypotheses: Nicotine Behavioral Sensitization.* **The hypothesis that nicotine behavioral sensitization would be greater in Lewis rats than in Fischer rats (Hypothesis 3A) was confirmed.** Lewis rats had more nicotine-induced locomotor activity than Fischer rats overall, although nicotine-induced locomotor activity peaked in non-stressed Fischer rats on Drug Day 5. **The hypothesis that stress would increase nicotine behavioral sensitization in Lewis and Fischer rats (Hypothesis 3B) was partially confirmed.** Stress increased nicotine-induced locomotor activity in Lewis rats, particularly on Drug Day 4, but decreased nicotine-induced locomotor activity in Fischer rats. **The hypothesis that effects of stress to increase nicotine behavioral sensitization would be greater in Lewis rats (Hypothesis 3C) was confirmed**, because nicotine-induced locomotor activity was increased in

Lewis rats but actually decreased in Fischer rats. In fact, nicotine-induced locomotor activity decreased in stressed Fischer rats to the point that nicotine behavioral sensitization did not occur in that group. **The hypothesis that impulsive action before drug administration would predict nicotine behavioral sensitization (Hypothesis 3D) was partially confirmed**, with baseline impulsive action predicting nicotine-induced locomotor activity on Drug Day 4, and interactions among rat strain, stress, and baseline impulsive action predicting nicotine-induced locomotor activity on several drug days. **The hypothesis that impulsive action before drug administration would predict nicotine behavioral sensitization to a greater degree in Lewis rats than in Fischer rats (Hypothesis 3E) was not confirmed**, as impulsive action was significantly correlated with activity in Fischer rats only on Drug Day 5.

*Impulsive action: Saline.* All rats were tested on the 5-CSRTT immediately following injections to determine the effect of saline injections on impulsive action. Rats were tested in two counterbalanced cohorts (A and B) on two separate, successive days of saline administration. In both Cohort A and Cohort B, there were no significant differences between stress or rat strain groups on Impulsive Action when saline was administered (see Table 24A in Appendix D).

*Impulsive Action: Nicotine Administration Days.* Impulsive action was greater in non-stressed rats than in stressed rats across all measured days [$F(1, 28.148) = 13.059$, $p < 0.01$]. Impulsive action also changed across the four days

measured [F(3, 46.625) = 4.339, p < 0.01], and changed differentially in stressed and non-stressed rats across days [F(3, 46.625) = 6.229, p < 0.01]. The mixed model analysis uses a regression to predict the estimated marginal means for each group on each day when there are missing datapoints (see estimated marginal means in Figure 18 and mixed model analysis in Table 25A in Appendix D).

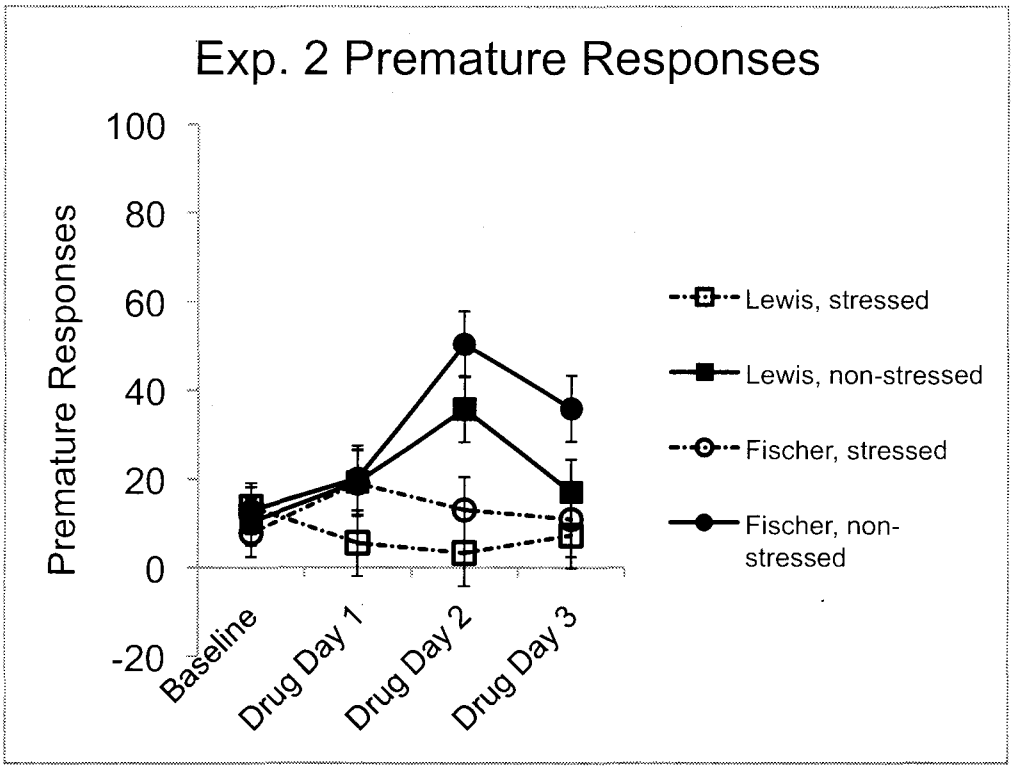



Figure 18. Premature responses (estimated marginal mean ± S.E.M) in all stress and rat strain groups across the four days measured.

*Summary: Impulsive Action.* Impulsive action was greater in the non-stressed groups than in the stressed groups, and changed differentially in stressed and non-stressed rats across the four days measured.

*Attention: Saline Days.* Neither Correct Responses nor Omissions differed among stress and rat strain groups on the saline days (see Tables 26A and 26B in Appendix D).

*Attention: Nicotine Administration Days.* Non-stressed rats had more correct responses than stressed rats [$F(1, 27.812) = 4.282$, $p < 0.05$], correct responses varied in the two rat strains across the four measured days [$F(3, 41.730) = 7.347$, $p < 0.001$], and correct responses varied by stress and rat strain groups across the days [$F(3, 41.730) = 4.002$, $p < 0.05$] (see estimated marginal means for correct responses in Figure 19 and analyses in Table 27A).

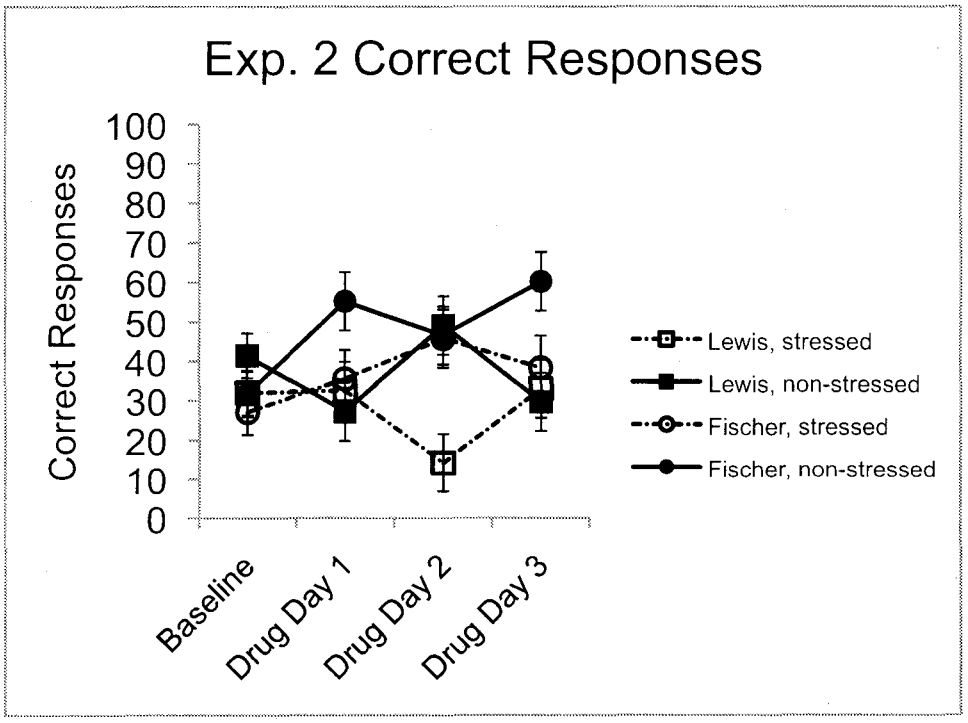



Figure 19. Correct responses (estimated marginal mean ± S.E.M.) in all stress and rat strain groups across the four days measured.

Omissions were greater in stressed rats than in non-stressed rats [$F(1, 26.938) = 4.996$, $p < 0.05$], were greater in Lewis rats than in Fischer rats [$F(1, 26.938) = 6.570$, $p < 0.05$], varied in the two rat strains across the four measured days [$F(3, 41.744) = 6.596$, $p < 0.01$], and varied by rat strain and stress group across the four measured days [$F(3, 41.744) = 3.244$, $p < 0.05$] (see Figure 20 and Table 28A in Appendix D).

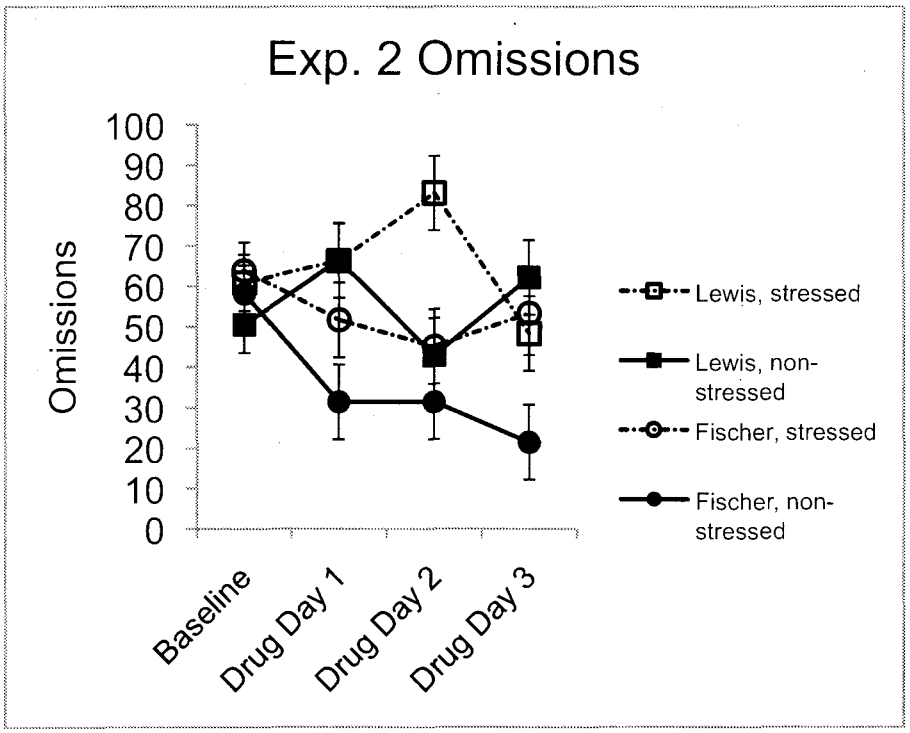



Figure 20. Omissions (estimated marginal mean ± S.E.M.) in all stress and rat strain groups across the four days measured.

*Summary: Attention.* There were no differences among stress and rat strain groups at the saline measurement. Attention varied by stress group and rat strain across the nicotine days that were measured. Both attention

parameters were improved during nicotine administration mainly in the Fischer non-stress group.

*Experiment 2 Summary.* As depicted in Table 6, the effects of stress differed in Lewis and Fischer rats on many of the dependent variables measured. Stressed rats had higher corticosterone levels than non-stressed rats in both rat strains. Fischer stressed rats had higher corticosterone levels than Fischer non-stressed rats and Lewis stressed rats, which had similar levels of corticosterone. Lewis non-stressed rats had the lowest level of corticosterone. Non-stressed Fischer rats had more nicotine-induced locomotor activity than stressed Fischer rats. Nicotine-induced locomotor activity was the greatest in Lewis stressed rats, which had more activity than Lewis non-stressed rats. Lewis non-stressed rats had a similar amount of activity to Fischer non-stressed rats, which had more activity than Fischer stressed rats.

During nicotine administration, stressed rats had less impulsive action than non-stressed rats in both rat strains. Fischer non-stressed rats had more impulsive action than Lewis non-stressed rats, which had more impulsive action than Fischer stressed rats, which had more impulsive action than Lewis stressed rats. Attention was detrimentally affected in both rat strains, with less correct responses and more omissions in stressed rats compared with non-stressed rats. The effects of stress to decrease attention were greatest in the Lewis rats.

| Dependent Variable | Lewis | Fischer | Lewis vs. Fischer |
|---|---|---|---|
| **Corticosterone** | S > NS * | S > NS * | FS > FNS = LS > LNS * |
| **Nicotine Behavioral Sensitization** | S ≥ NS | NS > S * | LS ≥ LNS ≥ FNS > FS * |
| **Impulsive Action** | S < NS * | S < NS * | FNS > LNS > FS > LS * |
| **Correct Responses** | S < NS * | S < NS * | FNS ≥ LNS ≥ FS ≥ LS * |
| **Omissions** | S > NS * | S > NS * | LS ≥ FS ≥ LNS > FNS * |

Table 6. Experiment 2 Effects. S indicates stress group, NS indicates non-stress group, L indicates Lewis rats, F indicates Fischer rats, and * indicates significance at $p > 0.05$.

# EXPERIMENT 2: ASSESSMENT AND DISCUSSION

## Assessment of Experiment 2 Hypotheses

**Hypothesis 2A:** Corticosterone. Stress will increase blood corticosterone levels in Lewis and Fischer rats: **supported.** Stress increased blood corticosterone levels in Lewis and Fischer rats.

**Hypothesis 2B:** Corticosterone and Rat Strain. Corticosterone will be elevated in stressed and non-stressed Fischer rats as compared with stressed and non-stressed Lewis rats: **supported.** Corticosterone levels were elevated in stressed and non-stressed Fischer rats as compared with stressed and non-stressed Lewis rats, respectively.

**Hypothesis 3A:** Nicotine Behavioral Sensitization. Behavioral sensitization to nicotine will be greater in Lewis rats than Fischer rats: **supported.** Nicotine-induced locomotor activity was greater in Lewis rats than in Fischer rats, and occurred in a pattern consistent with nicotine behavioral sensitization in stressed and non-stressed Lewis rats, and non-stressed Fischer rats. The pattern of nicotine-induced locomotor activity was not consistent with nicotine behavioral sensitization in Fischer stressed rats.

**Hypothesis 3B:** Stress and Nicotine Behavioral Sensitization. Psychological stress will increase behavioral sensitization to nicotine in Lewis and Fischer rats: **partially supported.** Psychological stress increased nicotine-induced locomotor activity in Lewis rats in a pattern consistent with nicotine behavioral sensitization. Psychological stress decreased

nicotine-induced locomotor activity in Fischer rats in a pattern that was inconsistent with nicotine behavioral sensitization.

**Hypothesis 3C:** Stress and Nicotine Behavioral Sensitization. The effect of stress to increase nicotine behavioral sensitization will be greater in Lewis rats than in Fischer rats: **partially supported.** Stress slightly increased nicotine behavioral sensitization in Lewis rats only, in a pattern consistent with nicotine behavioral sensitization. Stress decreased nicotine-induced locomotor activity in Fischer rats, in a pattern that was inconsistent with nicotine behavioral sensitization.

**Hypothesis 3D:** Baseline Impulsive Action and Nicotine Behavioral Sensitization. Impulsive action before drug administration will statistically predict behavioral sensitization to nicotine: **partially supported.** Baseline impulsive action predicted nicotine-induced locomotor activity on Drug Day 4 only.

**Hypothesis 3E:** Baseline Impulsive Action, Rat Strain, and Nicotine Behavioral Sensitization. Impulsive action before drug administration will statistically predict nicotine behavioral sensitization to a greater degree in Lewis rats than in Fischer rats: **not supported.** On Drug Day 4, the day on which baseline impulsive action predicted nicotine-induced locomotor activity, rat strain did not significantly contribute to the regression model. On Drug Days 5 and 6, baseline impulsive action predicted nicotine-induced locomotor activity in Fischer rats only.

**Hypothesis 4A:** Nicotine Behavioral Sensitization and Impulsive Action. Nicotine behavioral sensitization will increase impulsive action in Lewis and Fischer rats: **partially supported.** Impulsive action was increased in non-stressed rats during nicotine administration, consistent with an effect of nicotine behavioral sensitization on impulsive action. However, effects of nicotine cannot be separated from effects of time because a saline group was not included.

**Hypothesis 4B:** Nicotine Behavioral Sensitization, Stress, and Impulsive Action. Nicotine behavioral sensitization will increase impulsive action in stressed rats more than in non-stressed rats: **not supported.** Impulsive action was increased in non-stressed rats, but not stressed rats, during the nicotine administration phase.

**Hypothesis 4C:** Nicotine Behavioral Sensitization, Stress, Rat Strain, and Impulsive Action. The effect of nicotine behavioral sensitization to increase impulsive action will be greatest in Lewis stressed rats: **not supported.** Lewis stressed rats had the least premature responses of all groups on all nicotine administration days.

**Hypothesis 4D:** Nicotine Behavioral Sensitization and Attention. Nicotine will increase attention in the 5-CSRTT in Lewis and Fischer rats: **partially supported.** Nicotine administration increased attention mainly in Fischer non-stressed rats.

# DISCUSSION

## Experiment 2: The Effects of Stress and Impulsivity on Behavioral Sensitization to Nicotine

The primary goal of Experiment 2 was to determine the effects of stress and rat strain on nicotine behavioral sensitization, as indexed by locomotor activity. The secondary goal of Experiment 2 was to examine effects of nicotine behavioral sensitization on impulsive action and attention, as measured by the Five Choice Serial Reaction Time Task (5-CSRTT). The independent variables were psychological stress (stress, non-stress) and rat strain (Fischer, Lewis). Thirty-two male rats (n=8) were tested in a 2 (stress) x 2 (rat strain) factorial design with repeated measures. The dependent variables in the present research were nicotine-induced locomotor activity, impulsive action, and attention.

There were several findings in Experiment 2: (1) nicotine behavioral sensitization was greater in Lewis rats than Fischer rats; (2) psychological stress affected nicotine-induced locomotor activity differentially in Lewis and Fischer rats: stress increased nicotine-induced locomotor activity in Lewis rats and decreased nicotine-induced locomotor activity in Fischer rats; (3) baseline impulsive action (measured in Experiment 1) predicted nicotine-induced locomotor activity on Drug Day 4; (4) psychological stress decreased attention in Lewis and Fischer rats; (5) impulsive action was increased in non-stressed rats during nicotine administration; (6) nicotine administration increased attention, particularly in Fischer non-stressed rats. Each of these findings is discussed in

detail below, with regard to the context of the research literature and the implications of the findings. Consideration is given to relevant methodological issues and limitations of the present research.

**Finding # 1: Nicotine Behavioral Sensitization was greater in Lewis rats than Fischer rats.** When stress and non-stress groups were considered together within each rat strain, Lewis rats had more nicotine-induced locomotor activity than Fischer rats. The nicotine-induced locomotor activity occurred in a pattern that reflected nicotine behavioral sensitization in Lewis stressed and non-stressed rats, and Fischer non-stressed rats. The pattern of activity produced by nicotine in Fischer stressed rats did not reflect nicotine behavioral sensitization. Nicotine behavioral sensitization was pronounced and delayed in Fischer non-stressed rats, which was an unexpected pattern of results. With the exception of the previous, unpublished work, this experiment was the first to compare nicotine behavioral sensitization in Lewis and Fischer rats.

*Nicotine Reinforcement in Lewis and Fischer rats*

The finding that nicotine behavioral sensitization was greater in Lewis than Fischer rats is consistent with the previous work described in the introduction. The present finding also is consistent with previous reports of greater nicotine preference (Horan et al., 1997; Philibin et al., 2005), intake (Brower et al., 2002), and sensitivity (Philibin et al., 2005) in Lewis rats compared with Fischer rats. Nicotine behavioral sensitization is a manifestation of the incentive sensitization

phenomenon, and it indexes nicotine reinforcement (Stewart & Badiani, 1993; Vanderschuren & Kalivas, 2000, Robinson & Berridge, 1993; DiFranza & Wellman, 2007). Differences in nicotine reinforcement may be a mechanism underlying reported differences in nicotine intake, preference, and sensitivity (e.g., Philibin et al., 2005). If nicotine is more reinforcing for Lewis rats than Fischer rats, then it follows that greater nicotine reinforcement could lead to greater preference for, intake of, and sensitivity to the drug.

### *Pattern of Nicotine-Induced Activity Lewis and Fischer Rats: Relevance to Initiation*

The general pattern of nicotine-induced locomotor activity reported in the preliminary experiment also was reflected in the results of Experiment 2. In both experiments, nicotine-induced locomotor activity was maximal in the first 3-4 days of nicotine administration in Lewis rats. In the present experiment, nicotine-induced locomotor activity in Fischer non-stressed rats was maximal on the fifth day of nicotine administration. The results of both experiments suggest that nicotine is most reinforcing in the first few days of administration, and then becomes somewhat less reinforcing as administration continues.

Decreased nicotine reinforcement with continued nicotine administration is consistent with the Opponent Process Theory, or Counteradaptation (Solomon & Corbit, 1974). Counteradaptation occurs when the body attempts to overcome a euphoric state produced by drug administration with a counteracting aversive state that will return the body to a hedonic homeostasis. As the counteracting,

aversive state grows with experience and overcomes the effects of the euphoric state, tolerance to the drug occurs and the drug becomes less reinforcing. Koob and LeMoal (1997) proposed that both sensitization and counteradaptation occur as drug addiction develops. In the present research, it is likely that sensitization occurred during the first few days of nicotine administration, when locomotor activity was increasing. Decreasing locomotor activity in the later days of nicotine administration, indicating that the rats were less reinforced by nicotine, was likely a manifestation of counteradaptation.

The first few days of nicotine administration in an animal model are relevant to the initiation phase of cigarette smoking in humans, when a person is trying cigarettes for the first few times. The more reinforcing nicotine is during the initiation phase, the more likely an individual will be to continue to smoke. Lewis rats had higher baseline levels of impulsivity than Fischer rats in the present experiment. During the initiation phase in humans, higher levels of nicotine reinforcement in impulsive individuals may make them more likely to continue smoking, which may underlie reports of increased cigarette smoking in impulsive individuals (Mitchell, 1999; Bickell et al., 1999). In fact, initial sensitivity to nicotine reward and reinforcement in humans was associated with impulsive characteristics related to novelty seeking, response disinhibition, and extraversion (Perkins et al., 2008).

**Finding #2: Psychological stress affected nicotine-induced locomotor activity differentially in Lewis and Fischer rats: stress increased**

**nicotine-induced locomotor activity in Lewis rats and decreased nicotine-induced locomotor activity in Fischer rats.** Effects of stress on nicotine-induced locomotor activity were modest. Stress increased nicotine-induced locomotor activity in Lewis rats slightly on Drug Days 3 and 4, but decreased nicotine-induced locomotor activity in Fischer rats on Drug Day 5. While some effects of stress occurred, there were no main effects of stress on nicotine-induced locomotor activity in either Lewis or Fischer rats.

Effects of stress on nicotine-induced locomotor activity may have been modest for several reasons. First, stressors were counterbalanced across groups each day. Power may have been reduced by this procedure if some stressors were more effective at inducing stress than others. Second, no saline group was included in the present research, so it was not possible to examine a stress x drug interaction. Third, stress-group rats were also stressed during the first experiment. Prior experience with stress in Experiment 1 may have limited the effectiveness of stress induction in Experiment 2. However, despite these limitations, effects of stress differed by rat strain across nicotine administration days, and stressed Lewis rats had greater nicotine-induced locomotor activity than stressed Fischer rats.

### *Implications of Stress Effects on Nicotine Reinforcement*

Effects of stress on nicotine reinforcement imply that stress changes the extent to which individuals are reinforced by nicotine. Greater nicotine reinforcement under stress suggests that nicotine is more reinforcing to stressed

individuals than to non-stressed individuals. Conversely, decreased nicotine reinforcement under stress suggests that nicotine is less reinforcing to stressed individuals.

Similar to Experiment 1, effects of stress in Experiment 2 differed by rat strain. Stress increased nicotine reinforcement in Lewis rats, and decreased nicotine reinforcement in Fischer rats. Lewis rats were more impulsive than Fischer rats at baseline. It is possible that stress increases nicotine reinforcement in impulsive individuals, and decreases nicotine reinforcement in non-impulsive individuals. This possibility implies that impulsive individuals have an increased likelihood of initiating cigarette smoking during stress.

**Finding #3: Baseline impulsive action predicted nicotine-induced locomotor activity on Drug Day 4.** More baseline impulsive action was associated with more nicotine-induced locomotor activity. The fact that baseline impulsive action predicted nicotine-induced locomotor activity only on Drug Day 4 is of particular interest because nicotine-induced locomotor activity was maximal on Drug Day 4 in Lewis rats. On Drug Day 4, nicotine-induced locomotor activity was higher in the initially impulsive Lewis rat strain and was lower in the initially non-impulsive Fischer rat strain.

### *Nicotine Reinforcement and Nicotine Self-Administration*

The present finding that baseline impulsive action predicted nicotine-induced locomotor activity on Drug Day 4 is consistent with research in which

The prediction of nicotine-induced locomotor activity by baseline impulsive action lends strength to the extrapolation that impulsive individuals experience greater nicotine reinforcement, particularly during the initiation phase. The report that initial sensitivity to nicotine reward and reinforcement in humans was associated with impulsive characteristics related to novelty seeking, response disinhibition, and extraversion is consistent with this finding (Perkins et al., 2008).

**Finding #4: Psychological stress decreased attention in Lewis and Fischer rats.** Across the four days measured, psychological stress decreased attention. This effect was reflected in both attention parameters, correct responses and omissions. The effect of stress to decrease attention in Fischer and Lewis rats is consistent with effects of stress to decrease attention in Experiment 1, but inconsistent with effects of corticotrophin releasing factor (CRF) to increase attention reported by Ohmura et al. (2009). In the work by Ohmura et al. (2009), psychological stress *per se* was not manipulated. Experiments 1 and 2 are the first to manipulate psychological stress and examine its effects on attention in the 5-CSRTT.

*Effects of Stress and Nicotine on Attention*

In the present experiment, nicotine was administered to rats in both stress conditions. Rat strain differences emerged, with better attention in the Fischer rats than in the Lewis rats. While the same effect of rat strain occurred in Experiment 1, visual inspection of the data raises the possibility that the effect of

stress on attention in Fischer rats was somewhat mitigated by administration of nicotine. However, only inclusion of a saline group would have unequivocally revealed an effect of nicotine to mitigate effects of stress in Fischer rats.

**Finding # 5: Impulsive action was increased in non-stressed rats during nicotine administration.** Non-stressed rats had increased impulsive action during the nicotine phase compared to stressed rats, especially on Drug Day 2. Impulsive action changed in all stress groups across the four days measured, and was increased compared to baseline.

*Effects of Drug Administration on Impulsive Action*

Consistent with the present finding of increased impulsive action in non-stressed rats during the nicotine administration phase, acute nicotine administration increased impulsivity in rats in the 5-CSRTT (Blondel, Sanger, & Moser, 2000; Blondel, Simon, Sanger, & Moser, 1999; Mirza & Stolerman, 1998). Administration of other substances also induces premature responding. Phencylclidine (PCP) administration in rats (Amitai & Markou, 2009) and ethanol administration in mice (Oliver et al., 2009) increased impulsive action on the 5-CSRTT. Increased impulsive action in non-stressed rats during the nicotine administration phase also is consistent with reported increases in impulsive choice in rats on a delay discounting task after administration of acute and chronic nicotine (Dallery & Locey, 2005; Locey & Dallery, 2009). In addition, impulsive choice was increased after administration of cocaine (Anker et al.,

2009; Simon, Mendez, & Setlow, 2007) and morphine (Pattij, Schetters, Jannsen, Wiskerke, & Schoffelmeer, 2009), and after extended access to amphetamine self-administration (Gipson & Bardo, 2009). Because impulsive action and impulsive choice are related but dissociable, it is remarkable that both types of impulsivity are increased in rats by administration of various substances, including nicotine.

However, effects of nicotine administration on impulsive action cannot be separated from effects of time in the present research because no saline group was included. Despite this limitation, the pattern of impulsive action in non-stressed animals during nicotine administration was consistent with reported effects of nicotine on impulsive action (e.g., Blondel et al., 2003).

*Effects of Stress and Nicotine Administration on Impulsive Action*

While impulsive action was increased in non-stressed rats during the drug administration phase, impulsive action was not increased in stressed rats, and was particularly low in Lewis stressed rats. Low levels of impulsive action in Lewis stressed rats in the present experiment are consistent with low levels of impulsive action in Lewis stressed rats in Experiment 1. Levels of impulsive action in stressed Fischer rats also appear consistent with levels of impulsive action in stressed rats in Experiment 1. In contrast, levels of impulsive action were markedly elevated in non-stressed Fischer and Lewis rats in Experiment 2 compared to Experiment 1. Therefore, it appears that nicotine greatly increases impulsive action, and that psychological stress dampens the effect of nicotine on

impulsive action. However, without the inclusion of a saline control group, these conclusions cannot be made unequivocally.

**Finding #6: Attention was increased during nicotine administration, particularly in Fischer non-stressed rats.** On both attention parameters, correct responses and omissions, attention in Fischer non-stressed rats improved steadily across the nicotine administration days measured. Nicotine behavioral sensitization in Fischer non-stressed rats was not manifest until Drug Day 5, while Drug Day 3 was the last day on which attention was measured. However, it is possible that cognitive effects of nicotine also are sensitized by repeated nicotine administration and are manifested sooner than behavioral sensitization. Inclusion of a saline group would be needed to separate effects of nicotine from effects of time, such as practice effects.

In previous research, nicotine increased sustained attention or vigilance (Blondel et al., 2000) and enhanced selective attention (ability to ignore irrelevant stimuli) (Hahn et al., 2002) in the 5-CSRTT. The present results, especially in non-stressed Fischer rats, are consistent with this finding. However, inclusion of a saline group would be needed in order to unequivocally determine effects of nicotine administration on attention.

### *Limitations of Experiment 2*

There are some methodological limitations of Experiment 2 that limit the ability to make some conclusions unequivocally. First, rats were not randomly

assigned to stress condition in Experiment 2, but rather the same group assignments used in Experiment 1 also were used in Experiment 2. This means that it was not possible to separate effects of acute repeated stress from effects of prior stress exposure. However, in designing the experiment, this limitation was outweighed by the possibility that carryover effects of stress in Experiment 1 would contaminate behavioral responses of any non-stress rats in Experiment 2 that had been stressed in Experiment 1.

Second, the omission of a between-subjects saline group that received saline during the entire experiment limits the ability to unequivocally determine effects of nicotine behavioral sensitization on impulsive action and attention. Logistics were the limiting factor in this methodological decision. Inclusion of a saline group would have doubled the N of the experiment from 32 to 64. This increase in rats would have required an additional four months to train the second group daily on the 5-CSRTT, as only 32 rats could be trained per day. Because nicotine behavioral sensitization is a robust phenomenon that has been demonstrated in several experiments (e.g., Prus et al., 2008; DiFranza & Wellman, 2007), inclusion of a saline group was not needed to determine whether nicotine sensitization had occurred. Effects of stress and rat strain on impulsive action, attention, and nicotine behavioral sensitization were the primary focus of the present research, while effects of nicotine administration on impulsive action and attention were a secondary focus of the research. Therefore, non-stress control groups were included, but saline control-groups

were not included because the groups would have made the N prohibitively large.

# GENERAL DISCUSSION

Increased cigarette smoking under stress and increased cigarette smoking in impulsive individuals contribute to the tobacco epidemic in the United States, but the mechanisms underlying these relationships are unknown. Stress may make individuals more impulsive, or may make nicotine more reinforcing; either effect may drive increased cigarette smoking in stressed and impulsive individuals. The present experiments were conducted to determine whether stress affects impulsive action and reinforcing actions of nicotine differentially in impulsive and non-impulsive individuals, using a rat model of impulsivity. Each experiment had several major findings.

In Experiment 1, the effect of stress on impulsive action and attention was determined in Lewis (impulsive) and Fischer (non-impulsive) rats. Rats were measured on impulsive action in the Five-Choice Serial Reaction Time Task (5-CSRTT) at baseline (prior to stress induction), and for three days in which stress was induced with a combined immobilization restraint and predatory stress procedure in stress group rats prior to 5-CSRTT measurement. Lewis rats initially had greater impulsive action than Fischer rats, a finding that validates Lewis and Fischer rats as an animal model of impulsivity. However, stress changed the rank order of Lewis and Fischer rats on impulsivity: under stress, Lewis rats became less impulsive and Fischer rats became more impulsive. Attention was correlated with impulsive action in Experiment 1, and was decreased by stress.

In Experiment 2, the effect of stress on nicotine behavioral sensitization, which indexed nicotine reinforcement, was examined in Lewis (impulsive) and Fischer (non-impulsive) rats. Stress-group rats were stressed, and rats' locomotor activity was measured daily immediately after nicotine injections. Increased locomotor activity over time with repeated injections indicated nicotine behavioral sensitization. Nicotine behavioral sensitization was greater in Lewis rats than Fischer rats, and did not occur in Fischer stressed rats. Stress increased nicotine-induced locomotor activity in Lewis rats, and decreased nicotine-induced locomotor activity in Fischer rats. Further, baseline impulsive action predicted nicotine-induced locomotor activity on Day 4 of nicotine administration, the day in which nicotine-induced locomotor activity was maximal in Lewis rats. Lastly, stress decreased attention in Experiment 2, and repeated nicotine injections increased impulsive action in non-stressed rats. The present results are depicted in the Table 7 below.

| | **Baseline** | | | **Stress** | | | **Stress + Nicotine** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fischer** | | **Lewis** | **Fischer** | | **Lewis** | **Fischer** | | **Lewis** |
| **Impulsive Action** | Fischer | < | Lewis | ▲ Fischer | ≠ | ▼ Lewis | Fischer | > | Lewis |
| **Attention** | Fischer | = | Lewis | ▼ Fischer | ≠ | ▼ Lewis | Fischer | ≠ | Lewis |
| **Corticosterone** | NM | | NM | NM | | NM | Fischer | > | Lewis |
| **Nicotine Behavioral Sensitization** | NM | | NM | NM | | NM | Fischer | < | Lewis |

Table 7. Rat strain differences in impulsive action, attention, corticosterone, and nicotine behavioral sensitization in all phases. (NM = not measured)

The present results have several implications for impulsivity and nicotine reinforcement in impulsive and non-impulsive organisms. The implications of effects of stress on the rat model of impulsivity in the present research can be extrapolated to explain human smoking behavior under stress. A unified framework of implications relevant to human impulsivity and smoking behavior is proposed. Then, various explanations for the present results are discussed and conclusions drawn from the results follow.

## Effects of Stress on Impulsive Action and Nicotine Reinforcement

The mechanism by which stress increases cigarette smoking may differ in impulsive and non-impulsive individuals. The present results suggest that stress increases nicotine reinforcement, but not impulsive action, in impulsive organisms and increases impulsive action, but not nicotine reinforcement, in non-impulsive organisms. When extrapolated to humans, the present results imply that increased smoking behavior under stress in non-impulsive humans is caused by an increase in impulsivity, and increased smoking behavior under stress in impulsive individuals is caused by an increase in nicotine reinforcement.

The present research also addresses possible reasons that cigarette smoking is increased in impulsive individuals (e.g., Mitchell, 1999). While nicotine behavioral sensitization was increased in stressed Lewis rats, it also was high in Lewis non-stressed rats. At the same time, impulsive action was elevated in Lewis non-stressed rats. Therefore, not only are Lewis impulsive rats reinforced by nicotine, but they also have increased impulsive action. When

extrapolated to humans, it follows that two factors may be operating to increase the susceptibility of impulsive individuals to engage in cigarette smoking—an increased level of nicotine reinforcement and increased impulsivity.

In Fischer (non-impulsive) non-stressed rats, nicotine behavioral sensitization was robust, though somewhat delayed in its manifestation. When extrapolated to humans, non-impulsive individuals may be less likely to initiate cigarette smoking because they have a lower level of impulsive action, and a delayed onset of nicotine sensitization. Therefore, when trying cigarettes for the first time, non-impulsive individuals may not experience increased nicotine reinforcement until they have smoked several cigarettes, which would decrease their likelihood of initiating smoking. Additionally, non-impulsive individuals may be less likely to try cigarette smoking because of their low level of impulsivity. Fischer (non-impulsive) rats become impulsive when stressed. Extrapolating to humans, increased impulsivity under stress may make non-impulsive humans more likely to smoke cigarettes.

### *Impulsive Action and Stress in Lewis and Fischer Rats*

The relationship between impulsive action and stress in Lewis and Fischer rats can be represented as a U-shaped function. Initially, Lewis rats had more impulsive action than Fischer rats. When rats were stressed, impulsive action was decreased in Lewis rats and increased in Fischer rats. Therefore, stressed Fischer rats had more impulsive action than stressed Lewis rats.

Fischer rats are more stress sensitive than Lewis rats. In the present research, Fischer rats had higher levels of corticosterone than Lewis rats at the conclusion of the experiment. Stressed Fischer rats had the highest level of corticosterone, non-stressed Fischer rats and stressed Lewis rats had the same level of corticosterone, and non-stressed Lewis rats had the lowest level of corticosterone (Fischer stressed > Fischer non-stressed = Lewis stressed > Lewis non-stressed). Impulsive action changed as a function of stress level in a U-shaped curvilinear function, in which impulsive action is represented on the Y-axis and stress is represented on the X-axis. The relationship between stress and impulsive action in Lewis and Fischer rats is depicted in Figure 21.

LN= Lewis Non-Stressed Rats
LS= Lewis Stressed Rats
FN= Fischer Non-Stressed Rats
FS= Fischer Stressed Rats

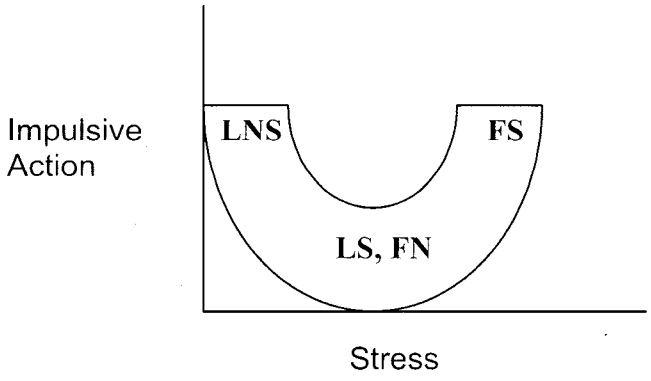



Figure 21. The relationship between impulsive action and stress in Lewis and Fischer stressed and non-stressed rats.

Impulsive action was greatest at the lowest (Lewis non-stressed) and highest (Fischer stressed) levels of stress. Impulsive action was decreased when stress was at a moderate level (Lewis stressed, Fischer non-stressed). The findings suggest that effects of stress on impulsive action in rats depend on level of initial (baseline) impulsive action and level of stress (level of corticosterone).

The U-shaped function representing the relationship between impulsive action and stress is based on the Yerkes-Dodson curve (1908), an inverted U-shaped function representing the relationship between arousal and performance. According to the Yerkes-Dodson principle, performance is enhanced as arousal increases, but too much arousal causes decrements in performance. The top of the inverted U-shaped function represents the optimal amount of arousal that causes the best performance. On the left side of the inverted U-shaped function, performance is enhanced as arousal increases. On the right side of the inverted U-shaped function, performance decreases as arousal increases.

The U-shaped function in Figure 21 is similar to the Yerkes-Dodson function (i.e., if the ordinate is reversed in Figure 21, then the function would be an inverted-U) in that impulsive action is increased at high and low levels of stress, but is decreased when levels of stress are moderate. Interestingly, this U-shaped function is only relevant to the relationship observed between impulsive action and stress that was observed in Experiment 1. When nicotine was administered to all rats in Experiment 2, impulsive action was augmented in non-stress rats such that the data no longer fit the U-shaped function. It is

possible that this function is mediated by a central mechanism in Lewis and Fischer rats, such as levels of CRF in the brain, or an interaction between CRF and dopamine levels in the brain. Obtaining daily or momentary assessments of corticosterone would be valuable to better characterize the relationships between stress and impulsive action, and stress and nicotine behavioral sensitization. One-time serum corticosterone samples were obtained from the euthanasia procedure at the conclusion of the present research. Daily samples of corticosterone could be obtained in a non-invasive manner by collecting feces to measure fecal corticosterone (Long, 2010; Cavigelli et al., 2005). Future research may elucidate the central mechanisms underlying the relationship between impulsive action and stress.

### *Conceptual Models*

While it is possible that one central mechanism mediates the relationship observed between impulsive action and stress, it is also possible that the causal mechanisms differ in Fischer and Lewis rats. The conceptual model with increased dopamine neurotransmission that was proposed in the introduction (page 35) seems only to fit the pattern of results for impulsive action observed in the Fischer rat strain. A modification of the existing conceptual model is required to describe the relationship between stress and nicotine behavioral sensitization in Fischer rats. In contrast, for the Lewis rat strain, the original conceptual model only describes the pattern of results observed for nicotine behavioral sensitization, but not for impulsive action. A modification of the existing

conceptual model is required to describe the relationship between stress and impulsive action in the Lewis rats strain. Therefore, two separate conceptual models of the effects of psychological stress on impulsive action and nicotine behavioral sensitization are depicted below for the Fischer (Figure 22) and Lewis (Figure 23) rat strains.

For the Fischer rats' conceptual model, increased dopamine neurotransmission mediates the relationship between stress, nicotine, and impulsive action, while a "black box" mediates the relationship between stress, nicotine, and nicotine behavioral sensitization. The effects of stress and nicotine on impulsive action observed in the present research in Fischer rats are consistent with that which would be predicted if increased dopamine neurotransmission were mediating the relationship. However, the effects of stress and nicotine on nicotine behavioral sensitization observed in Fischers were not consistent with that which would have been predicted if increased dopamine neurotransmission were mediating the relationship. For that reason, an alternative "black box" mechanism is proposed to mediate the relationship between stress, nicotine, and nicotine behavioral sensitization. The contents of the black box are not known, but it is possible that differences in nicotine pharmacokinetics (Sziraki et al., 2001), a lower level of nicotinic acetylcholinergic receptors, or a large increase in CRF in Fischer rats could mediate the relationship between stress, nicotine, and nicotine behavioral sensitization.

Figure 22. Conceptual Model: Fischer Rats

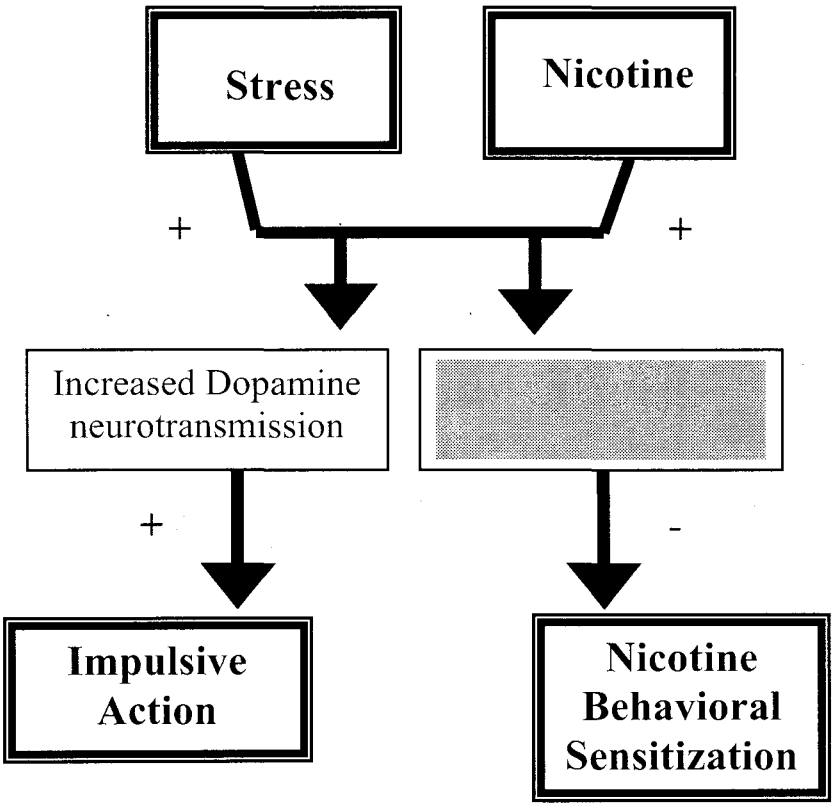
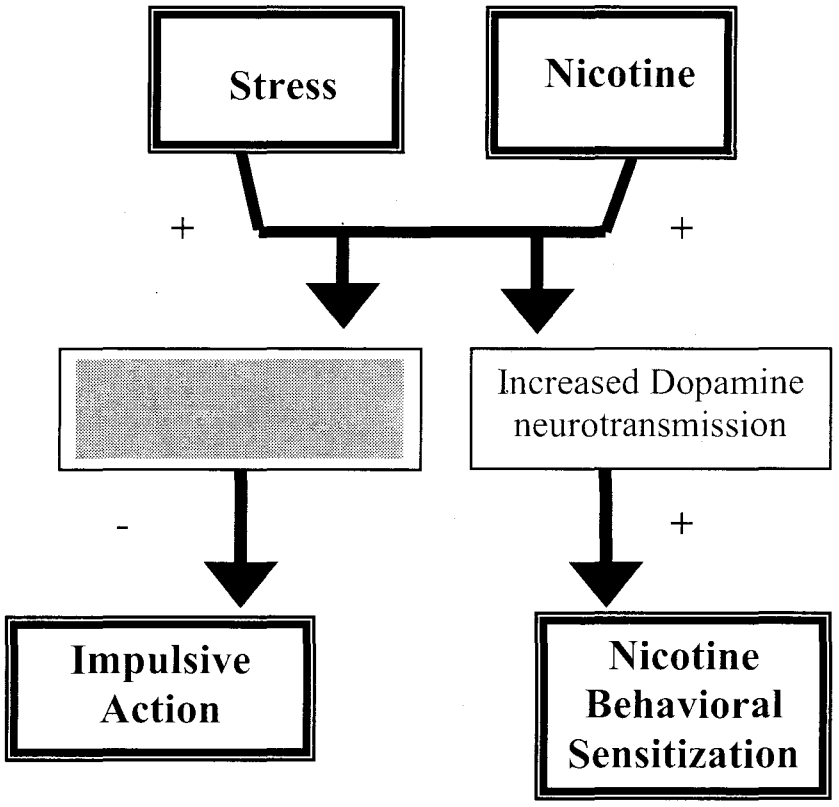


Figure 23. Conceptual Model: Lewis Rats




Similarly, for the Lewis rats' conceptual model, increased dopamine neurotransmission mediates the relationship between stress, nicotine, and nicotine behavioral sensitization, while a "black box" mediates the relationship between stress, nicotine, and impulsive action. The effects of stress and nicotine on nicotine behavioral sensitization observed in the present research in Fischer rats are consistent with that which would be predicted if increased dopamine neurotransmission were mediating the relationship. However, the effects of

stress and nicotine on impulsive action observed in Lewis rats were not consistent with that which would have been predicted if increased dopamine neurotransmission were mediating the relationship. For that reason, an alternative "black box" mechanism is proposed to mediate the relationship between stress, nicotine, and impulsive action. The contents of the black box are not known, but it is possible that a large increase in CRF or differential effects of stress on dopamine release in Lewis rats could mediate the relationship between stress, nicotine, and nicotine behavioral sensitization.

### *Law of Initial Values*

The Law of Initial Values can be used to explain biological rhythms (Wilder, 1962). According to this psychophysiological principle, the effect produced by a given stimulus tends to be the greatest when the initial value of a variable is low, and less of an effect is manifest when the initial value of a variable is high. Further, when initial values are extremely large, reversed or paradoxic responses frequently occur. A response to a stimulus is limited by its initial value because when more energy is initially exerted by an organism, the organism has less energy remaining to produce further increases in activity.

It is possible that the present results are consistent with the law of initial values. Lewis rats initially had high levels of impulsivity, while Fischer rats had low levels of impulsivity. After stress induction, impulsivity was decreased in Lewis rats and increased in Fischer rats. However, the Law of Initial Values would imply that a true effect of stress did not occur. It seems more likely that

the present impulsive action findings result from a true effect of stress for several reasons. First, the pattern of results was repeated across days of stress, with different types of stressors. If an effect of stress did not occur, then it is unlikely that the results would have been as consistent as they were. Second, effects of stress on attention, which was measured at the same time as impulsive action, were not consistent with the Law of Initial Values. Lewis rats initially had a higher level of attention than Fischer rats, but the effect of stress to decrease attention was greater in the Lewis rats than in the Fischer rats. If the results were consistent with the Law of Initial Values, then the greatest effect of stress would have occurred in the group with a lower baseline level of attention. If the law of initial values were acting on one type of measure, then its effects would have been evident on the other type of measure as well, because attention and impulsive action were measured at the same time. Third, effects of stress occurred on nicotine behavioral sensitization in a pattern that was not consistent with the Law of Initial Values. Effects of stress on nicotine behavioral sensitization occurred in the opposite direction in Lewis and Fischer rats, increasing nicotine-induced locomotor activity in Lewis rats and decreasing nicotine-induced locomotor-activity in Fischers. The opposite pattern of stress effects in each rat strain on impulsive action and nicotine-induced locomotor activity is not consistent with the Law of Initial Values, but rather suggests a true effect of stress on impulsive action that differs by rat strain.

*Baseline Impulsivity*

Baseline impulsivity has emerged from this research as an important factor influencing behavioral and pharmacological responses to various stimuli (i.e., stress, nicotine administration). In previous research (Anker et al., 2009; Barbelivien et al., 2007), effects of pharmacological manipulations on impulsivity depended on initial level of impulsivity. Results of the research of Anker and colleagues (2009) are consistent with the present results. Prior cocaine exposure increased impulsivity on a delay-discounting task in rats that initially had low levels of impulsivity, while level of impulsivity was unaffected in rats that initially had high levels of impulsivity (Anker et al., 2009). In the present research, stress increased impulsive action in rats with low levels of baseline impulsivity, and decreased impulsive action in rats with high levels of baseline impulsivity. In Experiment 2, reinforcing effects of nicotine also depended on baseline impulsivity.

Not only does baseline impulsivity influence the effect of stimuli on impulsivity, it also influences nicotine reinforcement. In human research, nicotine provided greater relief from negative affect in impulsive individuals (Doran et al., 2006). Therefore, impulsive individuals were more negatively reinforced by nicotine than non-impulsive individuals. In the present research, impulsive rats were more positively reinforced by nicotine than non-impulsive rats. Increased nicotine reinforcement in impulsive humans may increase their liability to smoke cigarettes. Baseline impulsivity also influences the development of nicotine dependence. Higher levels of nicotine dependence were associated with higher

levels of impulsive choice in current smokers, suggesting that impulsivity may be an important marker for vulnerability to develop nicotine dependence (Sweitzer, Donny, Dierker, Flory, & Manuck, 2008). Higher levels of nicotine dependence in impulsive individuals may have developed as a result of their increased nicotine reinforcement.

### *Attention and Impulsivity*

Attentional deficits and increased impulsivity co-occur in Attention Deficit Hyperactivity Disorder (ADHD), a co-occurrence that may suggest that attentional deficits are a component of impulsive behavior. In fact, de Wit (2009) proposed that attentional lapses may provide a dissociable, but related, measure of impulsivity.

The present results, however, suggest that attentional deficits do not necessarily translate into deficits in impulse control. In the present research, greater attention and less inattention were associated with impulsivity. This relationship was found most frequently in Lewis rats, and in stressed rats. Rather than inattention being a component of impulsivity, the present results suggest that attention is a component of impulsivity. Impulsivity in the 5-Choice Serial Reaction Time Task (5-CSRTT) is indexed by number of premature responses, which indicate response disinhibition. A premature response results from the unsuccessful inhibition of a prepotent response. Increased attention, or hypervigilance, may be the component of premature responses that contributes to their prepotency. Because the relationship between premature responses and

attention occurred most often in Lewis rats in the present research, it is likely that they are more hypervigilant than Fischer rats when monitoring the opportunity for reward.

Stress decreased attention in the 5-CSRTT in both the Lewis and Fischer rat strains, but the effect of stress to decrease attention was greatest in Lewis rats. Attention was positively correlated with impulsive action, and was likely involved in the emission of premature responses, especially in Lewis rats. When stress decreased attention, it also decreased impulsive action in Lewis rats.

Some of the stressed Lewis rats appeared not to be attending to the nose poke apertures during 5-CSRTT, demonstrating that a certain degree of attention is required to emit a premature response. Stress decreased attention and increased premature responses in Fischer rats. However, premature responses and correct responses were correlated less often in Fischer rats than in Lewis rats. Therefore, it is possible that the relationship between attention and impulsivity differs in Fischer and Lewis rats.

### *Impulsivity and Reinforcement Sensitivity*

Sensitivity to reinforcement may be elevated in impulsive individuals (Gray, 1970; Martin & Potts, 2004; Dawe, Gullo, & Loxton, 2004; Corr, 2004). Dawe et al. (2004) identified two dimensions of impulsivity as they relate to substance misuse: reward drive and rash impulsiveness. Dawe et al. (2004) proposed that a prepotent approach tendency must be inhibited or disinhibited in reward sensitivity, and that people with high levels of reward sensitivity will

experience stronger prepotent approach tendencies that would require greater levels of cognitive inhibition.

In the present research, impulsive Lewis rats may have had greater sensitivity to reward. Two findings of the present research support increased reinforcement sensitivity in impulsive individuals. First, nicotine reinforcement was elevated in impulsive rats. Nicotine-induced locomotor activity, which indexed reinforcement, was elevated in Lewis rats and was related to baseline impulsivity. Second, correct responses were positively correlated with impulsive action, suggesting that hypervigilance may have preceded the emission of premature responses. Enhanced reinforcement sensitivity may have been a motivating factor driving the relationship between impulsive action and attention.

### *The Role of Impulsivity in Drug Abuse*

Perry and Carroll (2008) proposed three non-mutually exclusive hypotheses about the role of impulsivity in drug use: (1) increased levels of impulsivity lead to drug abuse; (2) drugs of abuse increase impulsivity; (3) impulsivity and drug use are associated through a common third factor. The present experiments addressed Perry and Carroll's three hypotheses, and the results support each of the three hypotheses. With regard to the first hypothesis, greater reinforcing actions of nicotine in impulsives in the present research could lead to nicotine self-administration. The second hypothesis also was supported. Nicotine administration increased impulsive action in non-stressed Lewis and Fischer rats. Stress may be the common third factor proposed in the third

hypothesis. Stress impacted two factors that may lead to increased nicotine use differentially in impulsive and non-impulsive rats: impulsive action and reinforcing actions of nicotine. Therefore, each of Perry and Carroll's hypotheses about the role of impulsivity in drug abuse was supported. Impulsivity may increase drug abuse, drugs of abuse may increase impulsivity, and stress may be a common third factor with which impulsivity and drug use are associated.

## SUMMARY

The purpose of the present research was to determine the effect of stress on impulsive action, attention, and reinforcing actions of nicotine in a rat model of impulsivity, using Lewis and Fischer rats. In Lewis rats, stress decreased impulsive action and attention, and increased reinforcing actions of nicotine. In Fischer rats, stress decreased attention and reinforcing actions of nicotine, but increased impulsive action. Additionally, nicotine administration increased impulsive action in non-stressed rats. The present results are relevant to understanding psychological mechanisms underlying stress's effect to increase drug use.

## CONCLUSIONS

Several conclusions can be drawn from the present research: (1) Lewis and Fischer rats provide a valid rat model of impulsivity, with Lewis rats as the more impulsive rat strain. (2) Stress had an effect on impulsivity. Stress affected impulsivity differentially in Lewis (impulsive) and Fischer (non-impulsive) rats—

stress decreased impulsivity in Lewis rats and increased impulsivity in Fischer rats. (3) Stress decreased attention. (4) Reinforcing actions of nicotine were greater in impulsive than non-impulsive organisms. (5) Stress increased nicotine reinforcement in impulsive organisms and decreased nicotine reinforcement in non-impulsive organisms. The present results are relevant to why cigarette smoking is increased in impulsive and stressed individuals.

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# APPENDIX A

Timelines

Experiment 1

| Exp. Day | Procedure |
|---|---|
| 1-7 | Gentling, 5-CSRTT acclimation |
| 7-84 | 5-CSRTT Training |
| 85 | Locomotor Acclimation |
| 86 | Locomotor Testing |
| 87 | 5-CSRTT Testing |
| 88 | Stress Induction, 5-CSRTT Testing |
| 89 | Stress Induction, 5-CSRTT Testing |
| 90 | Stress Induction, 5-CSRTT Testing |
| 91 | Stress Induction, Locomotor Testing |

*5-CSRTT stands for Five Choice Serial Reaction Time Task

Experiment 2

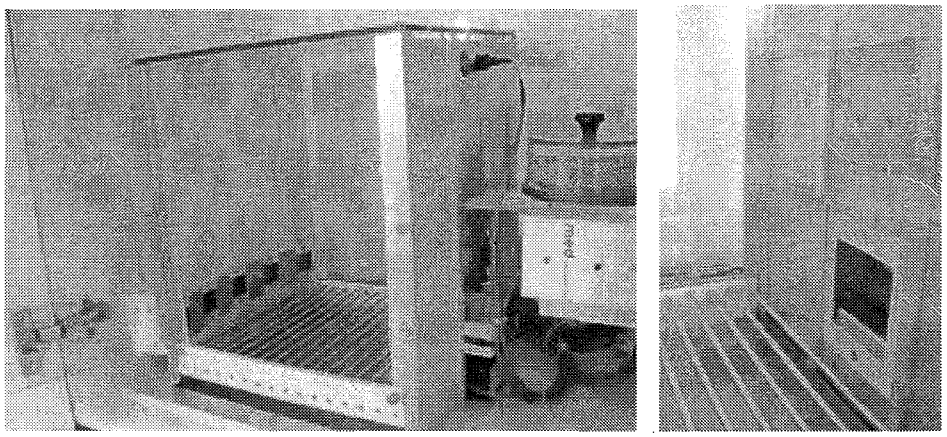
| Exp. Day | Procedure |
|---|---|
| 92 | Locomotor Testing |
| 93 | Baseline Saline, Locomotor Testing |
| 94 | Baseline Saline, Locomotor Testing, Coh. A 5-CSRTT Testing |
| 95 | Baseline Saline, Locomotor Testing, Coh. B 5-CSRTT Testing |
| 96 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. A 5-CSRTT* Testing |
| 97 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. B 5-CSRTT Testing |
| 98 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. A 5-CSRTT Testing |
| 99 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. B 5-CSRTT Testing |
| 100 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. A 5-CSRTT Testing |
| 101 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. B 5-CSRTT Testing |
| 102 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. A 5-CSRTT Testing |
| 103 | Stress, 0.5 mg/kg Nicotine, Locomotor Testing, Coh. B 5-CSRTT Testing |
| 104 | Stress, 5-CSRTT Testing |
| 105 | Stress, 5-CSRTT Testing |
| 106 | Stress, Locomotor Testing |

*5-CSRTT stands for Five Choice Serial Reaction Time Task

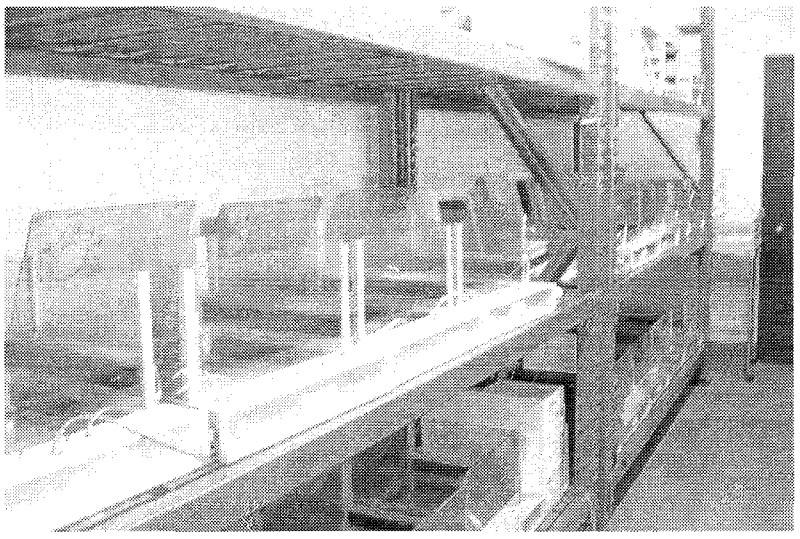
# APPENDIX B

Pictures of Equipment

Five Choice Serial Reaction Time Task (5-CSRTT)



Locomotor Chambers



# APPENDIX C

Data sheets

**Experiment One**
**Five Choice Testing**

**9/14/2009: No Stress**

**9/15/2009: Stress**

**9/16/2009: Stress**

**9/17/2009: Stress**

| I.D. | Box | 9/14/2009 | | 9/15/2009 | | 9/16/2009 | | 9/17/2009 | |
|---|---|---|---|---|---|---|---|---|---|
| 101 | 1 | | Inc: | | Inc: | | Inc: | | Inc: |
| | | | Om: | | Om: | | Om: | | Om: |
| | | | PR: | | PR: | | PR: | | PR: |
| 102 | 2 | | Inc: | | Inc: | | Inc: | | Inc: |
| | | | Om: | | Om: | | Om: | | Om: |
| | | | PR: | | PR: | | PR: | | PR: |
| 401 | 3 | | Inc: | | Inc: | | Inc: | | Inc: |
| | | | Om: | | Om: | | Om: | | Om: |
| | | | PR: | | PR: | | PR: | | PR: |
| 402 | 4 | | Inc: | | Inc: | | Inc: | | Inc: |
| | | | Om: | | Om: | | Om: | | Om: |
| | | | PR: | | PR: | | PR: | | PR: |

*"Inc" stands for Incorrect Responses, "Om" stands for omissions, and "PR" stands for premature responses.

Experiment Two
Stress Induction
Location G176

Monday, September 28, 2009
Stress Day One

| Cohort | Stress Cohort | Stressed Rat ID | Day 1 | completed |
|---|---|---|---|---|
| **1** | A1 | 401, 402 | Restraint stress + whistle | |
| **2** | A1 | 301, 302 | Restraint stress + whistle | |
| **3** | B1 | 403, 404 | Predator Stress + whistle | |
| **4** | B1 | 303, 304 | Predator Stress + whistle | |
| **5** | A2 | 405, 406 | Predator stress + cage shake | |
| **6** | A2 | 305, 306 | Predator stress + cage shake | |
| **7** | B2 | 407, 408 | Restraint stress + restrainer shake | |
| **8** | B2 | 307, 308 | Restraint stress + restrainer shake | |

**Supplies:**

16 mice cages with lids
16 cotton balls with fox urine in a plastic bag, set aside
8 restrainers
Whistle
Timer
Plastic bag
Reusable cloths
Alcohol solution spray

**Procedures:**

**Run 1:** A1—Restraint Stress and Whistle, B1—Predator Stress and Whistle

- 401, 402, 301, 302 (Cohorts 1 and 2) will be restrained for 20 minutes
- 403, 404, 303, 304 (Cohorts 3 and 4) will be in mice cages for 20 minutes.
  - At **10 minutes**, fox urine cotton balls will be dropped into the cages
- The **whistle** will be blown at **2 minutes, 6 minutes, 13 minutes, 15 minutes**, and **19 minutes**

**Run 2:** A2—Predator and Cage Shake, B2—Restraint Stress and Restrainer Shake

- 405, 406, 305, 306 (Cohorts 5 and 6) will be in mice cages for 20 minutes
  - At **10 minutes**, fox urine cotton balls will be dropped into the cages
- 407, 408, 307, 308 (Cohorts 7 and 8) will be in restrainers for 20 minutes
- Cages and restrainers will be **shaken** at **2 minutes, 6 minutes, 8 minutes, 13 minutes, 15 minutes**, and **19 minutes**

## Body Weight

| Rat ID | Date: | Date: | Date: | Date: | Date: |
|---|---|---|---|---|---|
| 101 | | | | | |
| 102 | | | | | |
| 401 | | | | | |
| 402 | | | | | |
| 201 | | | | | |
| 202 | | | | | |
| 301 | | | | | |
| 302 | | | | | |
| 103 | | | | | |
| 104 | | | | | |
| 403 | | | | | |
| 404 | | | | | |
| 203 | | | | | |
| 204 | | | | | |
| 303 | | | | | |
| 304 | | | | | |
| 105 | | | | | |
| 106 | | | | | |
| 405 | | | | | |
| 406 | | | | | |
| 205 | | | | | |
| 206 | | | | | |
| 305 | | | | | |
| 306 | | | | | |
| 107 | | | | | |
| 108 | | | | | |
| 407 | | | | | |
| 408 | | | | | |
| 207 | | | | | |
| 208 | | | | | |
| 307 | | | | | |
| 308 | | | | | |
| A | | | | | |
| B | | | | | |
| C | | | | | |
| D | | | | | |

## Free Feeders

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |

## **APPENDIX D:** Statistics Tables

*Experiment 1*

Table 1A: Baseline Impulsive Action

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 512 | 1 | 512 | 7.955 | 0.008 |
| **Error** | 1930.875 | 30 | 64.363 | | |

Table 1B.1: Lewis Baseline Impulsive Action

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Stress** | 7.563 | 1 | 7.563 | 0.065 | 0.803 |
| **Error** | 1636.875 | 14 | 116.920 | | |

Table 1B.2: Fischer Baseline Impulsive Action

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Stress** | 10.563 | 1 | 10.563 | 0.536 | 0.476 |
| **Error** | 275.875 | 14 | 19.705 | | |

Table 1C.1: Stress-group Baseline Impulsive Action

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 361 | 1 | 361 | 3.544 | 0.081 |
| **Error** | 1426 | 14 | 101.857 | | |

Table 1C.2: Non-stress group Baseline Impulsive Action

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 169 | 1 | 169 | 4.861 | 0.045 |
| **Error** | 486.75 | 14 | 34.768 | | |

Table 2A.1: All Stress Days Impulsive Action, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 22.904 | 2 | 11.452 | 0.449 | 0.641 |
| | Greenhouse-Geisser | 22.904 | 1.770 | 12.937 | 0.449 | 0.617 |
| **day * Premature** | Sphericity Assumed | 238.120 | 2 | 119.060 | 4.669 | 0.014 |
| | Greenhouse-Geisser | 238.120 | 1.770 | 134.498 | 4.669 | 0.017 |
| **day * Rat Strain** | Sphericity Assumed | 106.319 | 2 | 53.159 | 2.085 | 0.134 |
| | Greenhouse-Geisser | 106.319 | 1.770 | 60.052 | 2.085 | 0.141 |
| **day * Stress** | Sphericity Assumed | 103.584 | 2 | 51.792 | 2.031 | 0.141 |
| | Greenhouse-Geisser | 103.584 | 1.770 | 58.508 | 2.031 | 0.147 |
| **day * Rat Strain * Stress** | Sphericity Assumed | 20.909 | 2 | 10.454 | 0.410 | 0.666 |
| | Greenhouse-Geisser | 20.909 | 1.770 | 11.810 | 0.410 | 0.641 |
| **Error(day)** | Sphericity Assumed | 1376.963 | 54 | 25.499 | | |
| | Greenhouse-Geisser | 1376.963 | 47.802 | 28.806 | | |

Table 2A.2: All Stress Days Impulsive Action, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Premature** | 507.051 | 1 | 507.051 | 3.727 | 0.064 |
| **Rat Strain** | 380.359 | 1 | 380.359 | 2.795 | 0.106 |
| **Stress** | 55.836 | 1 | 55.836 | 0.410 | 0.527 |
| **Strain * Stress** | 1046.199 | 1 | 1046.199 | 7.689 | 0.010 |
| **Error** | 3673.741 | 27 | 136.065 | | |

Table 2B.1: Lewis All Stress Days Impulsive Action, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 34.209 | 2 | 17.105 | 0.958 | 0.397 |
| | Greenhouse-Geisser | 34.209 | 1.978 | 17.297 | 0.958 | 0.396 |
| **day * Premature** | Sphericity Assumed | 148.802 | 2 | 74.401 | 4.167 | 0.027 |
| | Greenhouse-Geisser | 148.802 | 1.978 | 75.239 | 4.167 | 0.027 |
| **day * Stress** | Sphericity Assumed | 31.236 | 2 | 15.618 | 0.875 | 0.429 |
| | Greenhouse-Geisser | 31.236 | 1.978 | 15.794 | 0.875 | 0.428 |
| **Error(day)** | Sphericity Assumed | 464.282 | 26 | 17.857 | | |
| | Greenhouse-Geisser | 464.282 | 25.710 | 18.058 | | |

Table 2B.2: Lewis All Stress Days Impulsive Action, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Premature** | 128.949 | 1.000 | 128.949 | 4.008 | 0.067 |
| **Stress** | 759.281 | 1.000 | 759.281 | 23.602 | 0.000 |
| **Error** | 418.218 | 13.000 | 32.171 | | |

Table 2B.3: Fischer All Stress Days Impulsive Action, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 23.864 | 2.000 | 11.932 | 0.351 | 0.707 |
| | Greenhouse-Geisser | 23.864 | 1.608 | 14.837 | 0.351 | 0.662 |
| **day * Premature** | Sphericity Assumed | 119.222 | 2.000 | 59.611 | 1.756 | 0.193 |
| | Greenhouse-Geisser | 119.222 | 1.608 | 74.125 | 1.756 | 0.200 |
| **day * Stress** | Sphericity Assumed | 92.137 | 2.000 | 46.068 | 1.357 | 0.275 |
| | Greenhouse-Geisser | 92.137 | 1.608 | 57.285 | 1.357 | 0.274 |
| **Error(day)** | Sphericity Assumed | 882.778 | 26.000 | 33.953 | | |
| | Greenhouse-Geisser | 882.778 | 20.909 | 42.220 | | |

Table 2B.4: Fischer All Stress Days Impulsive Action, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Premature** | 1000.574 | 1.000 | 1000.574 | 4.940 | 0.045 |
| **Stress** | 475.133 | 1.000 | 475.133 | 2.346 | 0.150 |
| **Error** | 2633.051 | 13.000 | 202.542 | | |

Table 2C.1: Stress Group All Stress Days Impulsive Action, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 24.496 | 2.000 | 12.248 | 0.373 | 0.692 |
| | Greenhouse-Geisser | 24.496 | 1.337 | 18.328 | 0.373 | 0.610 |
| **day * Premature** | Sphericity Assumed | 198.510 | 2.000 | 99.255 | 3.022 | 0.066 |
| | Greenhouse-Geisser | 198.510 | 1.337 | 148.525 | 3.022 | 0.091 |
| **day * Rat Strain** | Sphericity Assumed | 60.241 | 2.000 | 30.120 | 0.917 | 0.412 |
| | Greenhouse-Geisser | 60.241 | 1.337 | 45.072 | 0.917 | 0.380 |
| **Error(day)** | Sphericity Assumed | 853.907 | 26.000 | 32.843 | | |
| | Greenhouse-Geisser | 853.907 | 17.375 | 49.146 | | |

Table 2C.2: Stress Group All Stress Days Impulsive Action, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Premature** | 321.081 | 1.000 | 321.081 | 1.519 | 0.240 |
| **Rat Strain** | 1136.511 | 1.000 | 1136.511 | 5.377 | 0.037 |
| **Error** | 2747.877 | 13.000 | 211.375 | | |

Table 2C.3: Non-Stress Group All Stress Days Impulsive Action, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 13.675 | 2.000 | 6.837 | 0.383 | 0.686 |
| | Greenhouse-Geisser | 13.675 | 1.936 | 7.064 | 0.383 | 0.679 |
| **day * Premature** | Sphericity Assumed | 98.460 | 2.000 | 49.230 | 2.757 | 0.082 |
| | Greenhouse-Geisser | 98.460 | 1.936 | 50.862 | 2.757 | 0.084 |
| **day * Rat Strain** | Sphericity Assumed | 109.735 | 2.000 | 54.868 | 3.073 | 0.063 |
| | Greenhouse-Geisser | 109.735 | 1.936 | 56.686 | 3.073 | 0.065 |
| **Error(day)** | Sphericity Assumed | 464.206 | 26.000 | 17.854 | | |
| | Greenhouse-Geisser | 464.206 | 25.166 | 18.446 | | |

Table 2C.4: Non-Stress Group All Stress Days Impulsive Action, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Premature** | 195.096 | 1.000 | 195.096 | 2.767 | 0.120 |
| **Rat Strain** | 25.969 | 1.000 | 25.969 | 0.368 | 0.554 |
| **Error** | 916.737 | 13.000 | 70.518 | | |

3A: Baseline Correct Responses

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 892.531 | 1.000 | 892.531 | 3.719 | 0.063 |
| **Error** | 7199.688 | 30.000 | 239.990 | | |

3B: Baseline Omissions

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 561.125 | 1.000 | 561.125 | 1.655 | 0.208 |
| **Error** | 10170.875 | 30.000 | 339.029 | | |

4A.1: All Stress Days Correct Responses, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 1077.438 | 2.000 | 538.719 | 9.219 | 0.000 |
| | Greenhouse-Geisser | 1077.438 | 1.907 | 564.862 | 9.219 | 0.000 |
| **day * Rat Strain** | Sphericity Assumed | 95.813 | 2.000 | 47.906 | 0.820 | 0.446 |
| | Greenhouse-Geisser | 95.813 | 1.907 | 50.231 | 0.820 | 0.441 |
| **day * Stress** | Sphericity Assumed | 675.521 | 2.000 | 337.760 | 5.780 | 0.005 |
| | Greenhouse-Geisser | 675.521 | 1.907 | 354.151 | 5.780 | 0.006 |
| **day * Rat Strain * Stress** | Sphericity Assumed | 74.813 | 2.000 | 37.406 | 0.640 | 0.531 |
| | Greenhouse-Geisser | 74.813 | 1.907 | 39.222 | 0.640 | 0.524 |
| **Error(day)** | Sphericity Assumed | 3272.417 | 56.000 | 58.436 | | |
| | Greenhouse-Geisser | 3272.417 | 53.408 | 61.272 | | |

4A.2: All Stress Days Correct Responses, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 0.375 | 1.000 | 0.375 | 0.001 | 0.970 |
| **Stress** | 17712.667 | 1.000 | 17712.667 | 67.748 | 0.000 |
| **Strain * Stress** | 1944.000 | 1.000 | 1944.000 | 7.435 | 0.011 |
| **Error** | 7320.583 | 28.000 | 261.449 | | |

4B.1: Lewis All Stress Days Correct Responses, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 905.375 | 2.000 | 452.688 | 5.511 | 0.010 |
| | Greenhouse-Geisser | 905.375 | 1.686 | 537.147 | 5.511 | 0.014 |
| **day * Stress** | Sphericity Assumed | 320.792 | 2.000 | 160.396 | 1.953 | 0.161 |
| | Greenhouse-Geisser | 320.792 | 1.686 | 190.321 | 1.953 | 0.169 |
| **Error(day)** | Sphericity Assumed | 2299.833 | 28.000 | 82.137 | | |
| | Greenhouse-Geisser | 2299.833 | 23.597 | 97.461 | | |

4B.2: Lewis All Stress Days Correct Responses, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 34026.750 | 1.000 | 34026.750 | 126.867 | 0.000 |
| **Stress** | 15696.333 | 1.000 | 15696.333 | 58.523 | 0.000 |
| **Error** | 3754.917 | 14.000 | 268.208 | | |

4B.3: Fischer All Stress Days Correct Responses, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 267.875 | 2.000 | 133.938 | 3.856 | 0.033 |
| | Greenhouse-Geisser | 267.875 | 1.729 | 154.939 | 3.856 | 0.041 |
| **day * Stress** | Sphericity Assumed | 429.542 | 2.000 | 214.771 | 6.183 | 0.006 |
| | Greenhouse-Geisser | 429.542 | 1.729 | 248.447 | 6.183 | 0.009 |
| **Error(day)** | Sphericity Assumed | 972.583 | 28.000 | 34.735 | | |
| | Greenhouse-Geisser | 972.583 | 24.205 | 40.182 | | |

4B.4: Fischer All Stress Days Correct Responses, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 33708.000 | 1.000 | 33708.000 | 132.349 | 0.000 |
| **Stress** | 3960.333 | 1.000 | 3960.333 | 15.550 | 0.001 |
| **Error** | 3565.667 | 14.000 | 254.690 | | |

4C.1: Stress Group All Stress Days Correct Responses, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 1156.792 | 2.000 | 578.396 | 14.877 | 0.000 |
| | Greenhouse-Geisser | 1156.792 | 1.414 | 818.064 | 14.877 | 0.000 |
| **day * Rat Strain** | Sphericity Assumed | 2.625 | 2.000 | 1.313 | 0.034 | 0.967 |
| | Greenhouse-Geisser | 2.625 | 1.414 | 1.856 | 0.034 | 0.923 |
| **Error(day)** | Sphericity Assumed | 1088.583 | 28.000 | 38.878 | | |
| | Greenhouse-Geisser | 1088.583 | 19.797 | 54.988 | | |

4C.2: Stress Group All Stress Days Correct Responses, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 945.188 | 1.000 | 945.188 | 7.511 | 0.016 |
| **Error** | 1761.792 | 14.000 | 125.842 | | |

4C.3: Non-Stress Group All Stress Days Correct Responses, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 596.167 | 2.000 | 298.083 | 3.822 | 0.034 |
| | Greenhouse-Geisser | 596.167 | 1.993 | 299.161 | 3.822 | 0.034 |
| **day * Rat Strain** | Sphericity Assumed | 168.000 | 2.000 | 84.000 | 1.077 | 0.354 |
| | Greenhouse-Geisser | 168.000 | 1.993 | 84.304 | 1.077 | 0.354 |
| **Error(day)** | Sphericity Assumed | 2183.833 | 28.000 | 77.994 | | |
| | Greenhouse-Geisser | 2183.833 | 27.899 | 78.276 | | |

4C.4: Non-Stress Group All Stress Days Correct Responses, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 999.188 | 1.000 | 999.188 | 2.516 | 0.135 |
| **Error** | 5558.792 | 14.000 | 397.057 | | |

4D.1: All Stress Days Omissions, Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 1167.771 | 2.000 | 583.885 | 7.507 | 0.001 |
| | Greenhouse-Geisser | 1167.771 | 1.846 | 632.629 | 7.507 | 0.002 |
| **day * Rat Strain** | Sphericity Assumed | 197.021 | 2.000 | 98.510 | 1.267 | 0.290 |
| | Greenhouse-Geisser | 197.021 | 1.846 | 106.734 | 1.267 | 0.289 |
| **day * Stress** | Sphericity Assumed | 844.188 | 2.000 | 422.094 | 5.427 | 0.007 |
| | Greenhouse-Geisser | 844.188 | 1.846 | 457.330 | 5.427 | 0.009 |
| **day * Rat Strain * Stress** | Sphericity Assumed | 40.271 | 2.000 | 20.135 | 0.259 | 0.773 |
| | Greenhouse-Geisser | 40.271 | 1.846 | 21.816 | 0.259 | 0.755 |
| **Error(day)** | Sphericity Assumed | 4355.417 | 56.000 | 77.775 | | |
| | Greenhouse-Geisser | 4355.417 | 51.685 | 84.268 | | |

4D.2: All Stress Days Omissions, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 753.760 | 1.000 | 753.760 | 1.311 | 0.262 |
| **Stress** | 18900.094 | 1.000 | 18900.094 | 32.866 | 0.000 |
| **Rat Strain * Stress** | 4387.510 | 1.000 | 4387.510 | 7.630 | 0.010 |
| **Error** | 16101.958 | 28.000 | 575.070 | | |

4E.1: Lewis All Stress Days Omissions Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 1124.292 | 2.000 | 562.146 | 5.334 | 0.011 |
| | Greenhouse-Geisser | 1124.292 | 1.673 | 672.178 | 5.334 | 0.016 |
| **day * Stress** | Sphericity Assumed | 466.292 | 2.000 | 233.146 | 2.212 | 0.128 |
| | Greenhouse-Geisser | 466.292 | 1.673 | 278.781 | 2.212 | 0.138 |
| **Error(day)** | Sphericity Assumed | 2950.750 | 28.000 | 105.384 | | |
| | Greenhouse-Geisser | 2950.750 | 23.417 | 126.011 | | |

4E.1: Lewis All Stress Days Omissions Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 230464.083 | 1.000 | 230464.083 | 748.520 | 0.000 |
| **Stress** | 20750.083 | 1.000 | 20750.083 | 67.394 | 0.000 |
| **Error** | 4310.500 | 14.000 | 307.893 | | |

4E.3: Fischer All Stress Days Omissions Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 240.500 | 2.000 | 120.250 | 2.397 | 0.109 |
| | Greenhouse-Geisser | 240.500 | 1.476 | 162.938 | 2.397 | 0.127 |
| **day * Stress** | Sphericity Assumed | 418.167 | 2.000 | 209.083 | 4.168 | 0.026 |
| | Greenhouse-Geisser | 418.167 | 1.476 | 283.306 | 4.168 | 0.041 |
| **Error(day)** | Sphericity Assumed | 1404.667 | 28.000 | 50.167 | | |
| | Greenhouse-Geisser | 1404.667 | 20.664 | 67.975 | | |

4E.4: Fischer All Stress Days Omissions Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Stress** | 2537.521 | 1.000 | 2537.521 | 3.013 | 0.105 |
| **Error** | 11791.458 | 14.000 | 842.247 | | |

4F.1: Stress Group All Stress Days Omissions Within-Subjects Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 1190.167 | 2.000 | 595.083 | 9.509 | 0.001 |
| | Greenhouse-Geisser | 1190.167 | 1.369 | 869.389 | 9.509 | 0.003 |
| **day * Rat Strain** | Sphericity Assumed | 51.500 | 2.000 | 25.750 | 0.411 | 0.667 |
| | Greenhouse-Geisser | 51.500 | 1.369 | 37.620 | 0.411 | 0.592 |
| **Error(day)** | Sphericity Assumed | 1752.333 | 28.000 | 62.583 | | |
| | Greenhouse-Geisser | 1752.333 | 19.166 | 91.431 | | |

4F.2: Stress Group All Stress Days Omissions Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 311213.021 | 1.000 | 311213.021 | 515.449 | 0.000 |
| **Rat Strain** | 4389.188 | 1.000 | 4389.188 | 7.270 | 0.017 |
| **Error** | 8452.792 | 14.000 | 603.771 | | |

4F.3: Non-Stress Group All Stress Days Omissions Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 821.792 | 2.000 | 410.896 | 4.420 | 0.021 |
| | Greenhouse-Geisser | 821.792 | 1.969 | 417.265 | 4.420 | 0.022 |
| **day * Rat Strain** | Sphericity Assumed | 185.792 | 2.000 | 92.896 | 0.999 | 0.381 |
| | Greenhouse-Geisser | 185.792 | 1.969 | 94.336 | 0.999 | 0.380 |
| **Error(day)** | Sphericity Assumed | 2603.083 | 28.000 | 92.967 | | |
| | Greenhouse-Geisser | 2603.083 | 27.573 | 94.408 | | |

4F.4: Non-Stress Group All Stress Days Omissions Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 132090.083 | 1.000 | 132090.083 | 241.760 | 0.000 |
| **Rat Strain** | 752.083 | 1.000 | 752.083 | 1.377 | 0.260 |
| **Error** | 7649.167 | 14.000 | 546.369 | | |

5A.1: Correlations Between Baseline Impulsive Action and Correct Responses

| | | Correct | Premature |
|---|---|---|---|
| **Correct** | Pearson Correlation | 1.000 | 0.423 |
| | Sig. (2-tailed) | | 0.016 |
| | N | 32.000 | 32.000 |
| **Premature** | Pearson Correlation | 0.423 | 1.000 |
| | Sig. (2-tailed) | 0.016 | |
| | N | 32.000 | 32.000 |

5A.2: Correlations Between Baseline Impulsive Action and Omissions

| | | Premature | Omissions |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.00 | -0.51 |
| | Sig. (2-tailed) | | 0.00 |
| | N | 32.00 | 32.00 |
| **Omissions** | Pearson Correlation | -0.51 | 1.00 |
| | Sig. (2-tailed) | 0.00 | |
| | N | 32.00 | 32.00 |

5B.1: Lewis Correlations Between Baseline Impulsive Action and Correct Responses

| | | Premature | Correct |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | 0.379 |
| | Sig. (2-tailed) | | 0.148 |
| | N | 16.000 | 16.000 |
| **Correct** | Pearson Correlation | 0.379 | 1.000 |
| | Sig. (2- | 0.148 | |

| | | | |
|---|---|---|---|
| | Correlation | | |
| | Sig. (2-tailed) | | 0.058 |
| | N | 16.000 | 16.000 |
| **Omissions** | Pearson Correlation | -0.482 | 1.000 |
| | Sig. (2-tailed) | 0.058 | |
| | N | 16.000 | 16.000 |

5C.1: Stress Group Correlations Between Baseline Impulsive Action and Correct Responses

| | | **Premature** | **Correct** |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | 0.499 |
| | Sig. (2-tailed) | | 0.049 |
| | N | 16.000 | 16.000 |
| **Correct** | Pearson Correlation | 0.499 | 1.000 |
| | Sig. (2-tailed) | 0.049 | |
| | N | 16.000 | 16.000 |

5C.2: Stress Group Correlations Between Baseline Impulsive Action and Omissions

| | | **Premature** | **Omissions** |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | -0.499 |
| | Sig. (2-tailed) | | 0.049 |
| | N | 16.000 | 16.000 |
| **Omissions** | Pearson Correlation | -0.499 | 1.000 |
| | Sig. (2-tailed) | 0.049 | |
| | N | 16.000 | 16.000 |

5C.3: Non-Stress Group Correlations Between Baseline Impulsive Action and Correct Responses

| | | Premature | Correct |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | 0.408 |
| | Sig. (2-tailed) | | 0.117 |
| | N | 16.000 | 16.000 |
| **Correct** | Pearson Correlation | 0.408 | 1.000 |
| | Sig. (2-tailed) | 0.117 | |
| | N | 16.000 | 16.000 |

5C.4: Non-Stress Group Correlations Between Baseline Impulsive Action and Omissions

| | | Premature | Omissions |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | -0.636 |
| | Sig. (2-tailed) | | 0.008 |
| | N | 16.000 | 16.000 |
| **Omissions** | Pearson Correlation | -0.636 | 1.000 |
| | Sig. (2-tailed) | 0.008 | |
| | N | 16.000 | 16.000 |

6A.1: Correlations Between Stress Day 1 Impulsive Action and Correct Responses

| | | Str1 Correct | Str1 Premature |
|---|---|---|---|
| **Str1 Correct** | Pearson Correlation | 1.000 | 0.382 |
| | Sig. (2-tailed) | | 0.031 |
| | N | 32.000 | 32.000 |
| **Str1 Premature** | Pearson Correlation | 0.382 | 1.000 |
| | Sig. (2-tailed) | 0.031 | |
| | N | 32.000 | 32.000 |

* "Str1" refers to Stress Day 1

6A.1: Correlations Between Stress Day 1 Impulsive Action and Omissions

| | | Str1 Premature | Str1 Omissions |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | -0.416 |
| | Sig. (2-tailed) | | 0.018 |
| | N | 32.000 | 32.000 |
| **Str1 Omissions** | Pearson Correlation | -0.416 | 1.000 |
| | Sig. (2- | 0.018 | |

| | | | |
|---|---|---|---|
| | tailed) | | |
| | N | 32.000 | 32.000 |

6B.1: Lewis Correlations Between Stress Day 1 Impulsive Action and Correct Responses

| | | Str1 Premature | Str1 Correct |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | 0.789 |
| | Sig. (2-tailed) | | 0.000 |
| | N | 16.000 | 16.000 |
| **Str1 Correct** | Pearson Correlation | 0.789 | 1.000 |
| | Sig. (2-tailed) | 0.000 | |
| | N | 16.000 | 16.000 |

6B.2: Lewis Correlations Between Stress Day 1 Impulsive Action and Omissions

| | | Str1 Premature | Str1 Omissions |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | -0.804 |
| | Sig. (2-tailed) | | 0.000 |
| | N | 16.000 | 16.000 |
| **Str1 Omissions** | Pearson Correlation | -0.804 | 1.000 |
| | Sig. (2-tailed) | 0.000 | |
| | N | 16.000 | 16.000 |

6B.3: Fischer Correlations Between Stress Day 1 Impulsive Action and Correct Responses

| | | Str1 Premature | Str1 Correct |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1 | 0.0933 |
| | Sig. (2-tailed) | | 0.731 |
| | N | 16 | 16 |
| **Str1 Correct_Responses** | Pearson Correlation | 0.0933 | 1 |

| | | | |
|---|---|---|---|
| | Sig. (2-tailed) | 0.731 | |
| | N | 16 | 16 |

6B.4: Fischer Correlations Between Stress Day 1 Impulsive Action and Omissions

| | | **Str1 Premature** | **Str1 Omissions** |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | -0.120 |
| | Sig. (2-tailed) | | 0.659 |
| | N | 16.000 | 16.000 |
| **Str1 Omissions** | Pearson Correlation | -0.120 | 1.000 |
| | Sig. (2-tailed) | 0.659 | |
| | N | 16.000 | 16.000 |

6C.1: Stress Group Correlations Between Stress Day 1 Impulsive Action and Correct Responses

| | | **Str1 Premature** | **Str1 Correct** |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | 0.859 |
| | Sig. (2-tailed) | | 0.000 |
| | N | 16.000 | 16.000 |
| **Str1 Correct** | Pearson Correlation | 0.859 | 1.000 |
| | Sig. (2-tailed) | 0.000 | |
| | N | 16.000 | 16.000 |

6C.2: Stress Group Correlations Between Stress Day 1 Impulsive Action and Omissions

| | | **Str1 Premature** | **Str1 Omissions** |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | -0.442 |
| | Sig. (2-tailed) | | 0.087 |
| | N | 16.000 | 16.000 |

| | | | |
|---|---|---|---|
| **Str1 Omissions** | Pearson Correlation | -0.442 | 1.000 |
| | Sig. (2-tailed) | 0.087 | |
| | N | 16.000 | 16.000 |

6C.3: Non-Stress Group Correlations Between Stress Day 1 Impulsive Action and Correct Responses

| | | **Str1 Premature** | **Str1 Correct** |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | -0.098 |
| | Sig. (2-tailed) | | 0.718 |
| | N | 16.000 | 16.000 |
| **Str1 Correct** | Pearson Correlation | -0.098 | 1.000 |
| | Sig. (2-tailed) | 0.718 | |
| | N | 16.000 | 16.000 |

6C.4: Stress Group Correlations Between Stress Day 1 Impulsive Action and Omissions

| | | **Str1 Premature** | **Str1 Omissions** |
|---|---|---|---|
| **Str1 Premature** | Pearson Correlation | 1.000 | -0.103 |
| | Sig. (2-tailed) | | 0.703 |
| | N | 16.000 | 16.000 |
| **Str1** | Pearson | -0.103 | 1.000 |

| | | | |
|---|---|---|---|
| **Omissions** | Correlation | | |
| | Sig. (2-tailed) | 0.703 | |
| | N | 16.000 | 16.000 |

7A.1: Correlations Between Stress Day 2 Impulsive Action and Correct Responses

| | | **Str2 Premature** | **Str2Correct** |
|---|---|---|---|
| **Str2 Premature** | Pearson Correlation | 1.000 | 0.240 |
| | Sig. (2-tailed) | | 0.186 |
| | N | 32.000 | 32.000 |
| **Str2 Correct** | Pearson Correlation | 0.240 | 1.000 |
| | Sig. (2-tailed) | 0.186 | |
| | N | 32.000 | 32.000 |

7A.2: Correlations Between Stress Day 2 Impulsive Action and Omissions

| | | **Str2 Premature** | **Str2 Omissions** |
|---|---|---|---|
| **Str2 Premature** | Pearson Correlation | 1.000 | -0.300 |
| | Sig. (2-tailed) | | 0.096 |
| | N | 32.000 | 32.000 |
| **Str2** | Pearson | -0.300 | 1.000 |

| | | | |
|---|---|---|---|
| **Omissions** | Correlation | | |
| | Sig. (2-tailed) | 0.096 | |
| | N | 32.000 | 32.000 |

8A.1: Correlations Between Stress Day 3 Impulsive Action and Correct Responses

| | | **Str3 Correct** | **str3 Premature** |
|---|---|---|---|
| **Str3 Correct** | Pearson Correlation | 1.000 | 0.350 |
| | Sig. (2-tailed) | | 0.049 |
| | N | 32.000 | 32.000 |
| **Str3 Premature** | Pearson Correlation | 0.350 | 1.000 |
| | Sig. (2-tailed) | 0.049 | |
| | N | 32.000 | 32.000 |

8A.1: Correlations Between Stress Day 3 Impulsive Action and Omissions

| | | **Str3 Premature** | **Str3 Omissions** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | -0.470 |
| | Sig. (2-tailed) | | 0.007 |
| | N | 32.000 | 32.000 |
| **Str3** | Pearson | -0.470 | 1.000 |

| | | | |
|---|---|---|---|
| | tailed) | | |
| | N | 16.000 | 16.000 |

5B.2: Lewis Correlations Between Baseline Impulsive Action and Omissions

| | | **Premature** | **Omissions** |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | -0.507 |
| | Sig. (2-tailed) | | 0.045 |
| | N | 16.000 | 16.000 |
| **Omissions** | Pearson Correlation | -0.507 | 1.000 |
| | Sig. (2-tailed) | 0.045 | |
| | N | 16.000 | 16.000 |

5B.3: Fischer Correlations Between Baseline Impulsive Action and Correct Responses

| | | **Premature** | **Correct** |
|---|---|---|---|
| **Premature** | Pearson Correlation | 1.000 | 0.239 |
| | Sig. (2-tailed) | | 0.373 |
| | N | 16.000 | 16.000 |
| **Correct** | Pearson Correlation | 0.239 | 1.000 |
| | Sig. (2-tailed) | 0.373 | |
| | N | 16.000 | 16.000 |

5B.4: Fischer Correlations Between Baseline Impulsive Action and Omissions

| | | **Premature** | **Omissions** |
|---|---|---|---|
| **Premature** | Pearson | 1.000 | -0.482 |

| | | | |
|---|---|---|---|
| **Omissions** | Correlation | | |
| | Sig. (2-tailed) | 0.007 | |
| | N | 32.000 | 32.000 |

8B.1: Lewis Correlations Between Stress Day 3 Impulsive Action and Correct Responses

| | | **Str3 Premature** | **Str3 Correct** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | 0.723 |
| | Sig. (2-tailed) | | 0.002 |
| | N | 16.000 | 16.000 |
| **Str3 Correct** | Pearson Correlation | 0.723 | 1.000 |
| | Sig. (2-tailed) | 0.002 | |
| | N | 16.000 | 16.000 |

8B.2: Lewis Correlations Between Stress Day 3 Impulsive Action and Omissions

| | | **Str3 Premature** | **Str3 Omissions** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | -0.742 |
| | Sig. (2-tailed) | | 0.001 |
| | N | 16.000 | 16.000 |
| **Str3 Omissions** | Pearson Correlation | -0.742 | 1.000 |

| | | | |
|---|---|---|---|
| | Sig. (2-tailed) | 0.001 | |
| | N | 16.000 | 16.000 |

8B.3: Fischer Correlations Between Stress Day 3 Impulsive Action and Correct Responses

| | | **Str3 Premature** | **Str3 Correct** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | 0.119 |
| | Sig. (2-tailed) | | 0.660 |
| | N | 16.000 | 16.000 |
| **Str3 Correct** | Pearson Correlation | 0.119 | 1.000 |
| | Sig. (2-tailed) | 0.660 | |
| | N | 16.000 | 16.000 |

8B.4: Fischer Correlations Between Stress Day 3 Impulsive Action and Omissions

| | | **Str3 Prematre** | **Str3 Omissions** |
|---|---|---|---|
| **Str3 Prematre** | Pearson Correlation | 1.000 | -0.356 |
| | Sig. (2-tailed) | | 0.176 |

| | | | |
|---|---|---|---|
| | N | 16.000 | 16.000 |
| **Str3 Omissions** | Pearson Correlation | -0.356 | 1.000 |
| | Sig. (2-tailed) | 0.176 | |
| | N | 16.000 | 16.000 |

8C.1: Stress Group Correlations Between Stress Day 3 Impulsive Action and Correct Responses

| | | **Str3 Premature** | **Str3 Correct** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | 0.418 |
| | Sig. (2-tailed) | | 0.107 |
| | N | 16.000 | 16.000 |
| **Str3 Correct** | Pearson Correlation | 0.418 | 1.000 |
| | Sig. (2-tailed) | 0.107 | |
| | N | 16.000 | 16.000 |

8C.2: Stress Group Correlations Between Stress Day 3 Impulsive Action and Omissions

| | | **Str3 Premature** | **Str3 Omissions** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | -0.492 |
| | Sig. (2-tailed) | | 0.053 |

| | | | |
|---|---|---|---|
| | N | 16.000 | 16.000 |
| **Str3 Omissions** | Pearson Correlation | -0.492 | 1.000 |
| | Sig. (2-tailed) | 0.053 | |
| | N | 16.000 | 16.000 |

8C.3: Non-Stress Group Correlations Between Stress Day 3 Impulsive Action and Correct Responses

| | | **Str3 Premature** | **Str3 Correct** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | 0.580 |
| | Sig. (2-tailed) | | 0.019 |
| | N | 16.000 | 16.000 |
| **Str3 Correct** | Pearson Correlation | 0.580 | 1.000 |
| | Sig. (2-tailed) | 0.019 | |
| | N | 16.000 | 16.000 |

8C.4: Non-Stress Group Correlations Between Stress Day 3 Impulsive Action and Omissions

| | | **Str3 Premature** | **Str3 Omissions** |
|---|---|---|---|
| **Str3 Premature** | Pearson Correlation | 1.000 | -0.744 |
| | Sig. (2-tailed) | | 0.001 |
| | N | 16.000 | 16.000 |

| | | | |
|---|---|---|---|
| **Str3 Omissions** | Pearson Correlation | -0.744 | 1.000 |
| | Sig. (2-tailed) | 0.001 | |
| | N | 16.000 | 16.000 |

9A.1: Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 16482050.314 | 1.000 | 16482050.314 | 3.675 | 0.066 |
| | Greenhouse-Geisser | 16482050.314 | 1.000 | 16482050.314 | 3.675 | 0.066 |
| **day * rat strain** | Sphericity Assumed | 227473.391 | 1.000 | 227473.391 | 0.051 | 0.824 |
| | Greenhouse-Geisser | 227473.391 | 1.000 | 227473.391 | 0.051 | 0.824 |
| **day * stress** | Sphericity Assumed | 7645296.314 | 1.000 | 7645296.314 | 1.705 | 0.203 |
| | Greenhouse-Geisser | 7645296.314 | 1.000 | 7645296.314 | 1.705 | 0.203 |
| **day * rat strain * stress** | Sphericity Assumed | 1946350.160 | 1.000 | 1946350.160 | 0.434 | 0.516 |
| | Greenhouse-Geisser | 1946350.160 | 1.000 | 1946350.160 | 0.434 | 0.516 |
| **Error(day)** | Sphericity Assumed | 116609759.167 | 26.000 | 4484990.737 | | |
| | Greenhouse-Geisser | 116609759.167 | 26.000 | 4484990.737 | | |

9A.2: Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 10683850.160 | 1.000 | 10683850.160 | 2.021 | 0.167 |
| **stress** | 1073522.314 | 1.000 | 1073522.314 | 0.203 | 0.656 |
| **Rat strain * stress** | 1496068.776 | 1.000 | 1496068.776 | 0.283 | 0.599 |
| **Error** | 137441878.167 | 26.000 | 5286226.083 | | |

*Experiment Two*

10A.1: Corticosterone

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 1885694.076 | 1.000 | 1885694.076 | 38.647 | 0.000 |
| **Stress** | 1325726.000 | 1.000 | 1325726.000 | 27.171 | 0.000 |
| **Rat Strain * Stress** | 4131.972 | 1.000 | 4131.972 | 0.085 | 0.773 |
| **Error** | 1317403.743 | 27.000 | 48792.731 | | |

11A.1: Baseline Days and Saline Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 49086377.523 | 3.000 | 16362125.841 | 12.089 | 0.000 |
| | Greenhouse-Geisser | 49086377.523 | 2.570 | 19101169.110 | 12.089 | 0.000 |
| **day * rat strain** | Sphericity Assumed | 2951646.023 | 3.000 | 983882.008 | 0.727 | 0.539 |
| | Greenhouse-Geisser | 2951646.023 | 2.570 | 1148585.263 | 0.727 | 0.519 |
| **day * stress** | Sphericity Assumed | 8263257.398 | 3.000 | 2754419.133 | 2.035 | 0.115 |
| | Greenhouse-Geisser | 8263257.398 | 2.570 | 3215512.835 | 2.035 | 0.125 |
| **day * rat strain * stress** | Sphericity Assumed | 15796821.836 | 3.000 | 5265607.279 | 3.890 | 0.012 |
| | Greenhouse-Geisser | 15796821.836 | 2.570 | 6147077.469 | 3.890 | 0.017 |
| **Error(day)** | Sphericity Assumed | 113691261.969 | 84.000 | 1353467.404 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Greenhouse-Geisser | 113691261.969 | 71.955 | 1580039.784 | | |

11.A.2: Baseline Days and Saline Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 30951728.508 | 1.000 | 30951728.508 | 5.722 | 0.024 |
| **stress** | 17229183.758 | 1.000 | 17229183.758 | 3.185 | 0.085 |
| **Rat strain * stress** | 34274025.195 | 1.000 | 34274025.195 | 6.336 | 0.018 |
| **Error** | 151465094.156 | 28.000 | 5409467.648 | | |

11B.1: Lewis Baseline Days and Saline Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 23095128.047 | 3.000 | 7698376.016 | 6.792 | 0.001 |
| | Greenhouse-Geisser | 23095128.047 | 2.271 | 10167580.046 | 6.792 | 0.003 |
| **day * stress** | Sphericity Assumed | 4591159.547 | 3.000 | 1530386.516 | 1.350 | 0.271 |
| | Greenhouse-Geisser | 4591159.547 | 2.271 | 2021248.036 | 1.350 | 0.275 |
| **Error(day)** | Sphericity Assumed | 47604853.656 | 42.000 | 1133448.897 | | |
| | Greenhouse-Geisser | 47604853.656 | 31.800 | 1496995.257 | | |

11B.2: Lewis Baseline Days and Saline Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **stress** | 1451121.391 | 1.000 | 1451121.391 | 0.289 | 0.599 |
| **Error** | 70261093.469 | 14.000 | 5018649.533 | | |

11B.3: Fischer Baseline Days and Saline Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 28942895.500 | 3.000 | 9647631.833 | 6.131 | 0.001 |
| | Greenhouse-Geisser | 28942895.500 | 2.540 | 11397024.622 | 6.131 | 0.003 |
| **day * stress** | Sphericity Assumed | 19468919.688 | 3.000 | 6489639.896 | 4.124 | 0.012 |
| | Greenhouse-Geisser | 19468919.688 | 2.540 | 7666398.030 | 4.124 | 0.017 |
| **Error(day)** | Sphericity Assumed | 66086408.313 | 42.000 | 1573485.912 | | |
| | Greenhouse-Geisser | 66086408.313 | 35.553 | 1858804.108 | | |

11B.4: Fischer Baseline Days and Saline Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 4639040210.250 | 1.000 | 4639040210.250 | 799.795 | 0.000 |
| **stress** | 50052087.563 | 1.000 | 50052087.563 | 8.629 | 0.011 |
| **Error** | 81204000.688 | 14.000 | 5800285.763 | | |

11C.1: Stress Group Baseline Days and Saline Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 47029143.125 | 3.000 | 15676381.042 | 9.367 | 0.000 |
| | Greenhouse-Geisser | 47029143.125 | 2.424 | 19397664.591 | 9.367 | 0.000 |
| **day * rat strain** | Sphericity Assumed | 14993173.813 | 3.000 | 4997724.604 | 2.986 | 0.042 |
| | Greenhouse-Geisser | 14993173.813 | 2.424 | 6184092.191 | 2.986 | 0.055 |
| **Error(day)** | Sphericity Assumed | 70289894.063 | 42.000 | 1673568.906 | | |
| | Greenhouse-Geisser | 70289894.063 | 33.943 | 2070843.279 | | |

11C.2: Stress Group Baseline Days and Saline Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 3750521322.250 | 1.000 | 3750521322.250 | 713.997 | 0.000 |
| **Rat strain** | 42333.063 | 1.000 | 42333.063 | 0.008 | 0.930 |
| **Error** | 73539947.688 | 14.000 | 5252853.406 | | |

11C.3: Non-Stress Group Baseline Days and Saline Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 10320491.797 | 3.000 | 3440163.932 | 3.329 | 0.028 |
| | Greenhouse-Geisser | 10320491.797 | 2.686 | 3841796.604 | 3.329 | 0.034 |
| **day * rat strain** | Sphericity Assumed | 3755294.047 | 3.000 | 1251764.682 | 1.211 | 0.317 |
| | Greenhouse-Geisser | 3755294.047 | 2.686 | 1397905.856 | 1.211 | 0.317 |
| **Error(day)** | Sphericity Assumed | 43401367.906 | 42.000 | 1033365.903 | | |
| | Greenhouse-Geisser | 43401367.906 | 37.609 | 1154009.429 | | |

11C.4: Non-Stress Group Baseline Days and Saline Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Intercept** | 4503970210.141 | 1.000 | 4503970210.141 | 809.181 | 0.000 |
| **Rat strain** | 65183420.641 | 1.000 | 65183420.641 | 11.711 | 0.004 |
| **Error** | 77925146.469 | 14.000 | 5566081.891 | | |

12A: Baseline Day Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 10704564.500 | 1.000 | 10704564.500 | 3.135 | 0.088 |
| **stress** | 6612.500 | 1.000 | 6612.500 | 0.002 | 0.965 |
| **Rat strain * stress** | 24310.125 | 1.000 | 24310.125 | 0.007 | 0.933 |
| **Error** | 95600654.750 | 28.000 | 3414309.098 | | |

12B.1: Lewis Baseline Day Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **stress** | 2782.563 | 1.000 | 2782.563 | 0.001 | 0.978 |
| **Error** | 49041575.375 | 14.000 | 3502969.670 | | |

12B.1: Fischer Baseline Day Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| stress | 28140.063 | 1.000 | 28140.063 | 0.008 | 0.928 |
| Error | 46559079.375 | 14.000 | 3325648.527 | | |

12C.1: Stress Group Baseline Day Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Rat strain | 4854310.563 | 1.000 | 4854310.563 | 1.247 | 0.283 |
| Error | 54501567.375 | 14.000 | 3892969.098 | | |

12C.2: Non-Stress Group Baseline Day Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Rat strain | 5874564.063 | 1.000 | 5874564.063 | 2.001 | 0.179 |
| Error | 41099087.375 | 14.000 | 2935649.098 | | |

13.A: Saline Day 1 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Rat strain | 2459207.531 | 1.000 | 2459207.531 | 0.899 | 0.351 |
| stress | 3255714.031 | 1.000 | 3255714.031 | 1.190 | 0.285 |
| Rat strain * stress | 27712151.281 | 1.000 | 27712151.281 | 10.130 | 0.004 |
| Error | 76594656.375 | 28.000 | 2735523.442 | | |

13B.1: Lewis Saline Day 1 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| stress | 5985362.250 | 1.000 | 5985362.250 | 2.651 | 0.126 |
| Error | 31612713.500 | 14.000 | 2258050.964 | | |

13B.2: Fischer Saline Day 1 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **stress** | 24982503.063 | 1.000 | 24982503.063 | 7.775 | 0.015 |
| **Error** | 44981942.875 | 14.000 | 3212995.920 | | |

13C.1: Stress Group Saline Day 1 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 6830382.250 | 1.000 | 6830382.250 | 2.076 | 0.172 |
| **Error** | 46069035.500 | 14.000 | 3290645.393 | | |

13C.2: Non-Stress Group Saline Day 1 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 23340976.563 | 1.000 | 23340976.563 | 10.705 | 0.006 |
| **Error** | 30525620.875 | 14.000 | 2180401.491 | | |

14A: Saline Day 2 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 14764178.000 | 1.000 | 14764178.000 | 7.773 | 0.009 |
| **stress** | 5291004.500 | 1.000 | 5291004.500 | 2.786 | 0.106 |
| **Rat strain * stress** | 4051281.125 | 1.000 | 4051281.125 | 2.133 | 0.155 |
| **Error** | 53185177.250 | 28.000 | 1899470.616 | | |

14B.1: Lewis Saline Day 2 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **stress** | 41310.563 | 1.000 | 41310.563 | 0.026 | 0.874 |
| **Error** | 22132802.375 | 14.000 | 1580914.455 | | |

14B.2: Fischer Saline Day 2 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| stress | 9300975.063 | 1.000 | 9300975.063 | 4.193 | 0.060 |
| Error | 31052374.875 | 14.000 | 2218026.777 | | |

14C.1: Stress Group Saline Day 2 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Rat strain | 1673789.063 | 1.000 | 1673789.063 | 1.108 | 0.310 |
| Error | 21146784.875 | 14.000 | 1510484.634 | | |

14C.2: Non-Stress Group Saline Day 2 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Rat strain | 17141670.063 | 1.000 | 17141670.063 | 7.490 | 0.016 |
| Error | 32038392.375 | 14.000 | 2288456.598 | | |

15A: Saline Day 3 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Rat strain | 5975424.500 | 1.000 | 5975424.500 | 4.206 | 0.050 |
| stress | 16939110.125 | 1.000 | 16939110.125 | 11.924 | 0.002 |
| Rat strain * stress | 18283104.500 | 1.000 | 18283104.500 | 12.870 | 0.001 |
| Error | 39775867.750 | 28.000 | 1420566.705 | | |

15B.1: Lewis Saline Day 3 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| stress | 12825.563 | 1.000 | 12825.563 | 0.012 | 0.915 |
| Error | 15078855.875 | 14.000 | 1077061.134 | | |

15B.2: Fischer Saline Day 3 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **stress** | 35209389.063 | 1.000 | 35209389.063 | 19.959 | 0.001 |
| **Error** | 24697011.875 | 14.000 | 1764072.277 | | |

15C.1: Stress Group Saline Day 3 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 1677025.000 | 1.000 | 1677025.000 | 1.062 | 0.320 |
| **Error** | 22112454.000 | 14.000 | 1579461.000 | | |

15C.2: Non-Stress Group Saline Day 3 Locomotor Activity

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat strain** | 22581504.000 | 1.000 | 22581504.000 | 17.898 | 0.001 |
| **Error** | 17663413.750 | 14.000 | 1261672.411 | | |

16A: Nicotine Administration Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 130795203.395 | 7.000 | 18685029.056 | 2.568 | 0.016 |
| | Greenhouse-Geisser | 130795203.395 | 3.223 | 40575883.466 | 2.568 | 0.058 |
| **day * sal3ha** | Sphericity Assumed | 99540211.654 | 7.000 | 14220030.236 | 1.955 | 0.066 |
| | Greenhouse-Geisser | 99540211.654 | 3.223 | 30879817.634 | 1.955 | 0.126 |
| **day * rat strain** | Sphericity Assumed | 129786376.357 | 7.000 | 18540910.908 | 2.548 | 0.017 |
| | Greenhouse-Geisser | 129786376.357 | 3.223 | 40262920.550 | 2.548 | 0.060 |
| **day * stress** | Sphericity Assumed | 130346637.289 | 7.000 | 18620948.184 | 2.559 | 0.017 |
| | Greenhouse-Geisser | 130346637.289 | 3.223 | 40436727.247 | 2.559 | 0.059 |
| **day * rat** | Sphericity | 141009828.284 | 7.000 | 20144261.183 | 2.769 | 0.010 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **strain * stress** | Assumed | | | | | |
| | Greenhouse-Geisser | 141009828.284 | 3.223 | 43744710.904 | 2.769 | 0.045 |
| **Error(day)** | Sphericity Assumed | 967629877.034 | 133.000 | 7275412.609 | | |
| | Greenhouse-Geisser | 967629877.034 | 61.246 | 15799081.356 | | |

* "sal3ha" refers to the Saline Day 3 horizontal activity covariate

16.B: Nicotine Administration Days Locomotor Activity Between Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 15635382.377 | 1.000 | 15635382.377 | 0.438 | 0.516 |
| **Rat strain** | 180922623.598 | 1.000 | 180922623.598 | 5.066 | 0.036 |
| **stress** | 115209166.024 | 1.000 | 115209166.024 | 3.226 | 0.088 |
| **Rat strain * stress** | 75177526.819 | 1.000 | 75177526.819 | 2.105 | 0.163 |
| **Error** | 678606702.935 | 19.000 | 35716142.260 | | |

16.C: Lewis Nicotine Administration Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 225070540.772 | 7.000 | 32152934.396 | 6.635 | 0.000 |
| | Greenhouse-Geisser | 225070540.772 | 2.656 | 84747941.508 | 6.635 | 0.003 |
| **day * sal3ha** | Sphericity Assumed | 186321244.841 | 7.000 | 26617320.692 | 5.493 | 0.000 |
| | Greenhouse-Geisser | 186321244.841 | 2.656 | 70157302.263 | 5.493 | 0.007 |
| **day * stress** | Sphericity Assumed | 36195548.226 | 7.000 | 5170792.604 | 1.067 | 0.395 |
| | Greenhouse-Geisser | 36195548.226 | 2.656 | 13629052.445 | 1.067 | 0.376 |
| **Error(day)** | Sphericity Assumed | 305304434.596 | 63.000 | 4846102.136 | | |
| | Greenhouse-Geisser | 305304434.596 | 23.902 | 12773241.016 | | |

16.D: Lewis Nicotine Administration Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 17026352.723 | 1.000 | 17026352.723 | 1.031 | 0.337 |
| **stress** | 3101797.163 | 1.000 | 3101797.163 | 0.188 | 0.675 |
| **Error** | 148690789.589 | 9.000 | 16521198.843 | | |

16.E: Fischer Nicotine Administration Days Locomotor Activity Within-Subject

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 38788107.378 | 7.000 | 5541158.197 | 0.640 | 0.721 |
| | Greenhouse-Geisser | 38788107.378 | 2.306 | 16819147.516 | 0.640 | 0.559 |
| **day * sal3ha** | Sphericity Assumed | 29689203.414 | 7.000 | 4241314.773 | 0.490 | 0.839 |
| | Greenhouse-Geisser | 29689203.414 | 2.306 | 12873716.343 | 0.490 | 0.646 |
| **day * stress** | Sphericity Assumed | 187056863.410 | 7.000 | 26722409.059 | 3.084 | 0.007 |
| | Greenhouse-Geisser | 187056863.410 | 2.306 | 81110865.993 | 3.084 | 0.061 |
| **Error(day)** | Sphericity Assumed | 545855205.836 | 63.000 | 8664368.347 | | |
| | Greenhouse-Geisser | 545855205.836 | 20.756 | 26299066.762 | | |

16.F: Fischer Nicotine Administration Days Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 2883600.025 | 1.000 | 2883600.025 | 0.049 | 0.829 |
| **stress** | 80676439.750 | 1.000 | 80676439.750 | 1.381 | 0.270 |
| **Error** | 525641342.975 | 9.000 | 58404593.664 | | |

16.G: Stress Group Nicotine Administration Days Locomotor Activity Within-Subject Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 225947861.347 | 7.000 | 32278265.907 | 3.852 | 0.002 |
| | Greenhouse-Geisser | 225947861.347 | 3.101 | 72874398.542 | 3.852 | 0.019 |
| **day * sal3ha** | Sphericity Assumed | 185480736.731 | 7.000 | 26497248.104 | 3.162 | 0.006 |
| | Greenhouse-Geisser | 185480736.731 | 3.101 | 59822638.063 | 3.162 | 0.039 |
| **day * rat strain** | Sphericity Assumed | 279029367.716 | 7.000 | 39861338.245 | 4.757 | 0.000 |
| | Greenhouse-Geisser | 279029367.716 | 3.101 | 89994644.015 | 4.757 | 0.008 |
| **Error(day)** | Sphericity Assumed | 527945814.581 | 63.000 | 8380092.295 | | |
| | Greenhouse-Geisser | 527945814.581 | 27.905 | 18919671.443 | | |

16.H: Stress Group Nicotine Administration Days Locomotor Activity Between-Subjects Effects

| Source | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **day** | Sphericity Assumed | 16909986.023 | 7.000 | 2415712.289 | 0.438 | 0.875 |
| | Greenhouse-Geisser | 16909986.023 | 1.695 | 9975946.854 | 0.438 | 0.621 |
| **day * sal3ha** | Sphericity Assumed | 6355195.494 | 7.000 | 907885.071 | 0.165 | 0.991 |
| | Greenhouse-Geisser | 6355195.494 | 1.695 | 3749210.225 | 0.165 | 0.816 |
| **day * rat strain** | Sphericity Assumed | 65450624.544 | 7.000 | 9350089.221 | 1.696 | 0.126 |
| | Greenhouse-Geisser | 65450624.544 | 1.695 | 38612211.216 | 1.696 | 0.217 |
| **Error(day)** | Sphericity Assumed | 347388341.881 | 63.000 | 5514100.665 | | |
| | Greenhouse-Geisser | 347388341.881 | 15.256 | 22771078.918 | | |

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 130606021.022 | 1.000 | 130606021.022 | 7.097 | 0.026 |
| **Rat strain** | 383264929.361 | 1.000 | 383264929.361 | 20.826 | 0.001 |
| **Error** | 165628058.915 | 9.000 | 18403117.657 | | |

16.I: Non-Stress Group Nicotine Administration Days Locomotor Activity Within-Subject Effects

16.J: Non-Stress Group Nicotine Administration Days Locomotor Activity Between-Subject Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 32264333.741 | 1.000 | 32264333.741 | 0.794 | 0.396 |
| **Rat strain** | 58665500.076 | 1.000 | 58665500.076 | 1.444 | 0.260 |
| **Error** | 365743671.634 | 9.000 | 40638185.737 | | |

17.A: Drug Day 1 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 812840.997 | 1.000 | 812840.997 | 0.226 | 0.638 |
| **Rat strain** | 28294226.279 | 1.000 | 28294226.279 | 7.873 | 0.009 |
| **stress** | 149781.891 | 1.000 | 149781.891 | 0.042 | 0.840 |
| **Rat strain * stress** | 93673.707 | 1.000 | 93673.707 | 0.026 | 0.873 |
| **Error** | 97027974.753 | 27.000 | 3593628.695 | | |

17.B: Drug Day 2 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 240215.195 | 1.000 | 240215.195 | 0.082 | 0.776 |
| **Rat strain** | 15197766.171 | 1.000 | 15197766.171 | 5.217 | 0.030 |
| **stress** | 220417.817 | 1.000 | 220417.817 | 0.076 | 0.785 |

| | | | | | |
|---|---|---|---|---|---|
| **Rat strain * stress** | 271071.297 | 1.000 | 271071.297 | 0.093 | 0.763 |
| **Error** | 78653919.305 | 27.000 | 2913108.122 | | |

17.C: Drug Day 3 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 7772833.438 | 1.000 | 7772833.438 | 1.314 | 0.262 |
| **Rat strain** | 26577282.187 | 1.000 | 26577282.187 | 4.494 | 0.043 |
| **stress** | 24279691.534 | 1.000 | 24279691.534 | 4.105 | 0.053 |
| **Rat strain * stress** | 8084.688 | 1.000 | 8084.688 | 0.001 | 0.971 |
| **Error** | 159683507.812 | 27.000 | 5914203.993 | | |

17.D: Drug Day 4 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 81749693.021 | 1.000 | 81749693.021 | 3.809 | 0.064 |
| **Rat strain** | 127340036.673 | 1.000 | 127340036.673 | 6.518 | 0.018 |
| **stress** | 3774293.264 | 1.000 | 3774293.264 | 0.002 | 0.964 |
| **Rat strain * stress** | 61010565.476 | 1.000 | 61010565.476 | 2.157 | 0.156 |
| **Error** | 422662274.729 | 22.000 | 18376620.640 | | |

17.E: Drug Day 5 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 21145.896 | 1.000 | 21145.896 | 0.087 | 0.771 |
| **Rat strain** | 10401409.839 | 1.000 | 10401409.839 | 0.084 | 0.774 |
| **stress** | 127887813.075 | 1.000 | 127887813.075 | 2.381 | 0.137 |
| **Rat strain *** | 104215543.391 | 1.000 | 104215543.391 | 1.820 | 0.191 |

| | | | | | |
|---|---|---|---|---|---|
| **stress** | | | | | |
| **Error** | 618423100.354 | 22.000 | 26887960.885 | | |

17.F: Drug Day 6 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 286268.943 | 1.000 | 286268.943 | 0.038 | 0.847 |
| **Rat strain** | 15263008.009 | 1.000 | 15263008.009 | 2.015 | 0.167 |
| **stress** | 13033111.804 | 1.000 | 13033111.804 | 1.720 | 0.201 |
| **Rat strain * stress** | 4822851.463 | 1.000 | 4822851.463 | 0.637 | 0.432 |
| **Error** | 204545224.182 | 26.000 | 7575749.044 | | |

17.G: Drug Day 7 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 44317.451 | 1.000 | 44317.451 | 0.007 | 0.935 |
| **Rat strain** | 26619181.406 | 1.000 | 26619181.406 | 4.123 | 0.052 |
| **stress** | 2172469.485 | 1.000 | 2172469.485 | 0.336 | 0.567 |
| **Rat strain * stress** | 8117800.496 | 1.000 | 8117800.496 | 1.257 | 0.272 |
| **Error** | 174333196.174 | 26.000 | 6456785.043 | | |

17.H: Drug Day 8 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 564027.531 | 1.000 | 564027.531 | 0.079 | 0.780 |
| **Rat strain** | 8140340.788 | 1.000 | 8140340.788 | 1.144 | 0.294 |
| **stress** | 12563581.050 | 1.000 | 12563581.050 | 1.765 | 0.195 |
| **Rat strain * stress** | 7612563.770 | 1.000 | 7612563.770 | 1.070 | 0.310 |
| **Error** | 192178331.094 | 26.000 | 7117715.966 | | |

18.A: Lewis Drug Day 1 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 748016.027 | 1.000 | 748016.027 | 0.357 | 0.560 |
| **stress** | 11380.277 | 1.000 | 11380.277 | 0.005 | 0.942 |
| **Error** | 27212141.723 | 13.000 | 2093241.671 | | |

18.B: Fischer Drug Day 1 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 3312285.327 | 1.000 | 3312285.327 | 0.647 | 0.436 |
| **stress** | 1508782.120 | 1.000 | 1508782.120 | 0.295 | 0.596 |
| **Error** | 66568372.673 | 13.000 | 5120644.052 | | |

18.C: Lewis Drug Day 2 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 471469.348 | 1.000 | 471469.348 | 0.098 | 0.759 |
| stress | 688272.450 | 1.000 | 688272.450 | 0.143 | 0.711 |
| Error | 62437025.652 | 13.000 | 4802848.127 | | |

18.D: Fischer Drug Day 2 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 7305.621 | 1.000 | 7305.621 | 0.006 | 0.940 |
| stress | 68439.765 | 1.000 | 68439.765 | 0.056 | 0.817 |
| Error | 15978333.879 | 13.000 | 1229102.606 | | |

18.E: Lewis Drug Day 3 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 11778530.028 | 1.000 | 11778530.028 | 1.173 | 0.298 |
| stress | 16256569.213 | 1.000 | 16256569.213 | 1.619 | 0.225 |
| Error | 130506011.847 | 13.000 | 10038923.988 | | |

18.F: Fischer Drug Day 3 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 733549.566 | 1.000 | 733549.566 | 0.390 | 0.543 |
| stress | 2866516.390 | 1.000 | 2866516.390 | 1.525 | 0.239 |
| Error | 24438249.809 | 13.000 | 1879865.370 | | |

18.G: Lewis Drug Day 4 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 180307224.424 | 1.000 | 180307224.424 | 7.360 | 0.024 |
| stress | 20940089.069 | 1.000 | 20940089.069 | 0.855 | 0.379 |
| Error | 220481276.451 | 9.000 | 24497919.606 | | |

18.H: Fischer Drug Day 4 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 3452790.959 | 1.000 | 3452790.959 | 0.448 | 0.515 |
| stress | 2324263.045 | 1.000 | 2324263.045 | 0.302 | 0.592 |
| Error | 100170675.916 | 13.000 | 7705436.609 | | |

18.I: Lewis Drug Day 5 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 19739694.784 | 1.000 | 19739694.784 | 2.480 | 0.139 |
| stress | 1182359.513 | 1.000 | 1182359.513 | 0.149 | 0.706 |
| Error | 103469075.591 | 13.000 | 7959159.661 | | |

18.J: Fischer Drug Day 5 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 17032347.860 | 1.000 | 17032347.860 | 0.321 | 0.585 |
| stress | 187492948.381 | 1.000 | 187492948.381 | 3.529 | 0.093 |
| Error | 478203128.015 | 9.000 | 53133680.891 | | |

18.K: Lewis Drug Day 6 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 2173959.886 | 1.000 | 2173959.886 | 0.820 | 0.382 |
| stress | 1451997.933 | 1.000 | 1451997.933 | 0.548 | 0.472 |
| Error | 34467344.864 | 13.000 | 2651334.220 | | |

18.L: Fischer Drug Day 6 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 223810.176 | 1.000 | 223810.176 | 0.017 | 0.897 |
| stress | 14804862.120 | 1.000 | 14804862.120 | 1.146 | 0.304 |
| Error | 167966378.199 | 13.000 | 12920490.631 | | |

18.M: Lewis Drug Day 7 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 186452.512 | 1.000 | 186452.512 | 0.088 | 0.772 |
| stress | 1375127.103 | 1.000 | 1375127.103 | 0.647 | 0.436 |
| Error | 27621652.363 | 13.000 | 2124742.489 | | |

18.N: Fischer Drug Day 7 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 365496.422 | 1.000 | 365496.422 | 0.032 | 0.860 |
| stress | 7280415.867 | 1.000 | 7280415.867 | 0.647 | 0.436 |
| Error | 146203912.328 | 13.000 | 11246454.794 | | |

18.O: Lewis Drug Day 8 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 116111.186 | 1.000 | 116111.186 | 0.060 | 0.811 |
| stress | 374607.580 | 1.000 | 374607.580 | 0.193 | 0.668 |
| Error | 25290419.689 | 13.000 | 1945416.899 | | |

18.P: Fischer Drug Day 8 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 1486824.779 | 1.000 | 1486824.779 | 0.117 | 0.738 |
| stress | 8725628.556 | 1.000 | 8725628.556 | 0.684 | 0.423 |
| Error | 165849002.971 | 13.000 | 12757615.613 | | |

19.A: Stress Group Drug Day 1 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 751736.386 | 1.000 | 751736.386 | 0.412 | 0.532 |
| **Rat strain** | 13931745.051 | 1.000 | 13931745.051 | 7.631 | 0.016 |
| **Error** | 23733873.489 | 13.000 | 1825682.576 | | |

19.B: Non-Stress Group Drug Day 1 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 5396441.487 | 1.000 | 5396441.487 | 1.032 | 0.328 |
| **Rat strain** | 1462055.533 | 1.000 | 1462055.533 | 0.280 | 0.606 |
| **Error** | 67958764.388 | 13.000 | 5227597.261 | | |

19.C: Stress Group Drug Day 2 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 2344505.266 | 1.000 | 2344505.266 | 0.719 | 0.412 |
| **Rat strain** | 7458392.389 | 1.000 | 7458392.389 | 2.288 | 0.154 |
| **Error** | 42382501.734 | 13.000 | 3260192.441 | | |

19.D: Non-Stress Group Drug Day 2 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 955920.070 | 1.000 | 955920.070 | 0.374 | 0.551 |
| **Rat strain** | 10434868.503 | 1.000 | 10434868.503 | 4.085 | 0.064 |
| **Error** | 33211207.430 | 13.000 | 2554708.264 | | |

19.E: Stress Group Drug Day 3 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 61635.469 | 1.000 | 61635.469 | 0.067 | 0.800 |
| **Rat strain** | 18602453.471 | 1.000 | 18602453.471 | 20.129 | 0.001 |
| **Error** | 12014175.906 | 13.000 | 924167.377 | | |

19.F: Non-Stress Group Drug Day 3 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 19904920.640 | 1.000 | 19904920.640 | 1.910 | 0.190 |
| **Rat strain** | 21079638.663 | 1.000 | 21079638.663 | 2.023 | 0.179 |
| **Error** | 135475609.235 | 13.000 | 10421200.710 | | |

19.G: Stress Group Drug Day 4 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 161199450.540 | 1.000 | 161199450.540 | 5.677 | 0.035 |
| **Rat strain** | 311920205.816 | 1.000 | 311920205.816 | 9.747 | 0.009 |
| **Error** | 319849760.335 | 13.000 | 24603827.718 | | |

19.H: Non-Stress Group Drug Day 4 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 2220556.660 | 1.000 | 2220556.660 | 0.945 | 0.356 |
| Rat strain | 40001873.805 | 1.000 | 40001873.805 | 17.028 | 0.003 |
| Error | 21142200.215 | 9.000 | 2349133.357 | | |

19.I: Stress Group Drug Day 5 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 2285832.530 | 1.000 | 2285832.530 | 0.018 | 0.898 |
| Rat strain | 104352377.461 | 1.000 | 104352377.461 | 1.928 | 0.202 |
| Error | 169313759.970 | 9.000 | 18812639.997 | | |

19.J: Non-Stress Group Drug Day 5 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 2261912.197 | 1.000 | 2261912.197 | 0.066 | 0.801 |
| Rat strain | 15661059.408 | 1.000 | 15661059.408 | 0.458 | 0.510 |
| Error | 444582741.553 | 13.000 | 34198672.427 | | |

19.K: Stress Group Drug Day 6 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 7711296.959 | 1.000 | 7711296.959 | 1.060 | 0.322 |
| Rat strain | 30342773.637 | 1.000 | 30342773.637 | 4.169 | 0.062 |
| Error | 94616588.416 | 13.000 | 7278199.109 | | |

19.L: Non-Stress Group Drug Day 6 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 15287493.559 | 1.000 | 15287493.559 | 2.279 | 0.155 |

| | | | | | |
|---|---|---|---|---|---|
| **Rat strain** | 11435286.360 | 1.000 | 11435286.360 | 1.704 | 0.214 |
| **Error** | 87216114.191 | 13.000 | 6708931.861 | | |

19.M: Stress Group Drug Day 7 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 5791028.845 | 1.000 | 5791028.845 | 0.625 | 0.443 |
| **Rat strain** | 45107235.257 | 1.000 | 45107235.257 | 4.868 | 0.046 |
| **Error** | 120455312.905 | 13.000 | 9265793.300 | | |

19.N: Non-Stress Group Drug Day 7 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 5648289.371 | 1.000 | 5648289.371 | 1.728 | 0.211 |
| **Rat strain** | 8207716.779 | 1.000 | 8207716.779 | 2.512 | 0.137 |
| **Error** | 42482882.504 | 13.000 | 3267914.039 | | |

19.O: Stress Group Drug Day 8 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 3476543.322 | 1.000 | 3476543.322 | 0.343 | 0.568 |
| **Rat strain** | 25022610.730 | 1.000 | 25022610.730 | 2.472 | 0.140 |
| **Error** | 131595741.428 | 13.000 | 10122749.341 | | |

19.P: Non-Stress Group Drug Day 8 Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 10324603.44 | 1 | 10324603.44 | 2.8349 | 0.1161 |
| **Rat strain** | 3624831.885 | 1 | 3624831.885 | 0.9953 | 0.3367 |
| **Error** | 47345470.43 | 13 | 3641959.264 | | |

20.A: Post-Drug Day Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **sal3ha** | 1339766.429 | 1.000 | 1339766.429 | 0.186 | 0.670 |
| **Rat strain** | 12567025.246 | 1.000 | 12567025.246 | 1.746 | 0.197 |
| **stress** | 53293689.062 | 1.000 | 53293689.062 | 7.403 | 0.011 |
| **Rat strain * stress** | 1294598.626 | 1.000 | 1294598.626 | 0.180 | 0.675 |
| **Error** | 194362853.696 | 27.000 | 7198624.211 | | |

20.B: Lewis Post-Drug Day Locomotor Activity Between-Subjects Effects

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 10906500.233 | 1.000 | 10906500.233 | 2.646 | 0.114 |
| | Residual | 123662479.767 | 30.000 | 4122082.659 | | |
| | Total | 134568980.000 | 31.000 | | | |

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 7975333.176 | 1.000 | 7975333.176 | 1.632 | 0.224 |
| stress | 26298509.398 | 1.000 | 26298509.398 | 5.382 | 0.037 |
| Error | 63526208.199 | 13.000 | 4886631.400 | | |

20.C: Fischer Post-Drug Day Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 544247.589 | 1.000 | 544247.589 | 0.057 | 0.815 |
| stress | 11002821.498 | 1.000 | 11002821.498 | 1.157 | 0.302 |
| Error | 123656831.161 | 13.000 | 9512063.935 | | |

20.D: Stress Group Post-Drug Day Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 4765245.041 | 1.000 | 4765245.041 | 0.838 | 0.377 |
| Rat strain | 15050456.618 | 1.000 | 15050456.618 | 2.648 | 0.128 |
| Error | 73897983.334 | 13.000 | 5684460.256 | | |

20.E: Non-Stress Group Post-Drug Day Locomotor Activity Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| sal3ha | 497716.544 | 1.000 | 497716.544 | 0.056 | 0.817 |
| Rat strain | 5032506.678 | 1.000 | 5032506.678 | 0.561 | 0.467 |
| Error | 116541675.206 | 13.000 | 8964744.247 | | |

21A: Baseline Impulsive Action and Drug Day 1 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | Regression | 1722787.610 | 1.000 | 1722787.610 | 0.531 | 0.472 |
| | Residual | 97258908.265 | 30.000 | 3241963.609 | | |
| | Total | 98981695.875 | 31.000 | | | |

21B: Baseline Impulsive Action and Drug Day 2 Horizontal Activity Regression

21C: Baseline Impulsive Action and Drug Day 3 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 418696.961 | 1.000 | 418696.961 | 0.061 | 0.807 |
| | Residual | 206519676.914 | 30.000 | 6883989.230 | | |
| | Total | 206938373.875 | 31.000 | | | |

21D: Baseline Impulsive Action and Drug Day 4 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 375879090.805 | 1.000 | 375879090.805 | 22.464 | 0.000 |
| | Residual | 435038698.624 | 26.000 | 16732257.639 | | |
| | Total | 810917789.429 | 27.000 | | | |

21E: Baseline Impulsive Action and Drug Day 5 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 16388540.706 | 1.000 | 16388540.706 | 0.466 | 0.501 |
| | Residual | 914650853.151 | 26.000 | 35178878.967 | | |
| | Total | 931039393.857 | 27.000 | | | |

21F: Baseline Impulsive Action and Drug Day 6 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 3348732.721 | 1.000 | 3348732.721 | 0.405 | 0.529 |
| | Residual | 248155756.748 | 30.000 | 8271858.558 | | |
| | Total | 251504489.469 | 31.000 | | | |

21G: Baseline Impulsive Action and Drug Day 7 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 11144798.905 | 1.000 | 11144798.905 | 1.605 | 0.215 |
| | Residual | 208280353.313 | 30.000 | 6942678.444 | | |
| | Total | 219425152.219 | 31.000 | | | |

21H: Baseline Impulsive Action and Drug Day 8 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 9392589.376 | 1.000 | 9392589.376 | 1.235 | 0.275 |
| | Residual | 228084705.592 | 30.000 | 7602823.520 | | |
| | Total | 237477294.969 | 31.000 | | | |

21 I: Baseline Impulsive Action and Post-Drug Day 3 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 31128538.910 | 1.000 | 31128538.910 | 3.790 | 0.061 |
| | Residual | 246380616.058 | 30.000 | 8212687.202 | | |
| | Total | 277509154.969 | 31.000 | | | |

22 A: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 1 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 38431237.240 | 3.000 | 12810412.413 | 3.731 | 0.023 |
| | Residual | 96137742.760 | 28.000 | 3433490.813 | | |
| | Total | 134568980.000 | 31.000 | | | |

22 B: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 2 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 20143131.471 | 3.000 | 6714377.157 | 2.385 | 0.090 |
| | Residual | 78838564.404 | 28.000 | 2815663.014 | | |
| | Total | 98981695.875 | 31.000 | | | |

22 C: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 3 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 29348969.281 | 3.000 | 9782989.760 | 1.542 | 0.225 |
| | Residual | 177589404.594 | 28.000 | 6342478.735 | | |
| | Total | 206938373.875 | 31.000 | | | |

22 D: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 4 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 481329447.119 | 3.000 | 160443149.040 | 11.683 | 0.000 |
| | Residual | 329588342.310 | 24.000 | 13732847.596 | | |
| | Total | 810917789.429 | 27.000 | | | |

22 E: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 5 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 322616468.995 | 3.000 | 107538822.998 | 4.242 | 0.015 |
| | Residual | 608422924.863 | 24.000 | 25350955.203 | | |
| | Total | 931039393.857 | 27.000 | | | |

22 F: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 6 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 63443013.014 | 3.000 | 21147671.005 | 3.149 | 0.041 |
| | Residual | 188061476.455 | 28.000 | 6716481.302 | | |
| | Total | 251504489.469 | 31.000 | | | |

22 G: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 7 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 53158749.729 | 3.000 | 17719583.240 | 2.984 | 0.048 |
| | Residual | 166266402.500 | 28.000 | 5938085.804 | | |
| | Total | 219425152.229 | 31.000 | | | |

22 H: Baseline Impulsive Action and Rat Strain Interaction, Drug Day 8 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 50529596.337 | 3.000 | 16843198.779 | 2.523 | 0.078 |
| | Residual | 186947698.632 | 28.000 | 6676703.523 | | |
| | Total | 237477294.969 | 31.000 | | | |

22 I: Baseline Impulsive Action and Rat Strain Interaction, Post-Drug Day 3 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 35555149.864 | 3.000 | 11851716.621 | 1.372 | 0.272 |
| | Residual | 241954005.104 | 28.000 | 8641214.468 | | |
| | Total | 277509154.969 | 31.000 | | | |

23A: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 1 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 55816372.652 | 7.000 | 7973767.522 | 2.430 | 0.049 |
| | Residual | 78752607.348 | 24.000 | 3281358.639 | | |
| | Total | 134568980.000 | 31.000 | | | |

23B: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 2 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 23041221.228 | 7.000 | 3291603.033 | 1.040 | 0.430 |
| | Residual | 75940474.647 | 24.000 | 3164186.444 | | |
| | Total | 98981695.875 | 31.000 | | | |

23C: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 3 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 110336111.158 | 7.000 | 15762301.594 | 3.916 | 0.006 |
| | Residual | 96602262.717 | 24.000 | 4025094.280 | | |
| | Total | 206938373.875 | 31.000 | | | |

23D: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 4 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 554062999.660 | 7.000 | 79151857.094 | 6.163 | 0.001 |
| | Residual | 256854789.769 | 20.000 | 12842739.488 | | |
| | Total | 810917789.429 | 27.000 | | | |

23E: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 5 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 526980836.527 | 7.000 | 75282976.647 | 3.726 | 0.010 |
| | Residual | 404058557.330 | 20.000 | 20202927.867 | | |
| | Total | 931039393.857 | 27.000 | | | |

23F: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 6 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 116523472.958 | 7.000 | 16646210.423 | 2.960 | 0.022 |
| | Residual | 134981016.511 | 24.000 | 5624209.021 | | |
| | Total | 251504489.469 | 31.000 | | | |

23G: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 7 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 99456061.254 | 7.000 | 14208008.751 | 2.842 | 0.026 |
| | Residual | 119969090.965 | 24.000 | 4998712.124 | | |
| | Total | 219425152.219 | 31.000 | | | |

23H: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Drug Day 8 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 88890495.413 | 7.000 | 12698642.202 | 2.051 | 0.090 |
| | Residual | 148586799.556 | 24.000 | 6191116.648 | | |
| | Total | 237477294.969 | 31.000 | | | |

23I: Baseline Impulsive Action, Rat Strain, and Stress Interaction, Post-Drug Day 3 Horizontal Activity Regression

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 98092871.437 | 7.000 | 14013267.348 | 1.875 | 0.119 |
| | Residual | 179416283.532 | 24.000 | 7475678.481 | | |
| | Total | 277509154.969 | 31.000 | | | |

24A: Impulsive Action During Saline Administration, Between-Subjects Effects

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 22.781 | 1.000 | 22.781 | 0.255 | 0.618 |
| **Stress** | 5.281 | 1.000 | 5.281 | 0.059 | 0.810 |
| **Rat** | 148.781 | 1.000 | 148.781 | 1.663 | 0.208 |

| | | | | | |
|---|---|---|---|---|---|
| **Strain * Stress** | | | | | |
| **Error** | 2505.375 | 28.000 | 89.478 | | |

25A: Impulsive Action Across Saline Day and Drug Days 1, 2, and 3, Mixed Model Analysis

| Source | Numerator df | Denominator df | F | Sig. |
|---|---|---|---|---|
| **Rat Strain** | 1.000 | 28.148 | 2.981 | 0.095 |
| **Stress** | 1.000 | 28.148 | 13.059 | 0.001 |
| **DrugDay** | 3.000 | 46.625 | 4.339 | 0.009 |
| **Rat Strain * Stress** | 1.000 | 28.148 | 0.230 | 0.635 |
| **Rat Strain * DrugDay** | 3.000 | 46.625 | 1.461 | 0.237 |
| **Stress * DrugDay** | 3.000 | 46.625 | 6.229 | 0.001 |
| **Rat Strain * Stress * DrugDay** | 3.000 | 46.625 | 0.861 | 0.468 |

26A: Attention During Saline Administration, Between-Subjects Effects, Correct Responses

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| **Rat Strain** | 435.125 | 1.000 | 435.125 | 1.613 | 0.215 |
| **Stress** | 392.000 | 1.000 | 392.000 | 1.453 | 0.238 |
| **Rat Strain * Stress** | 50.000 | 1.000 | 50.000 | 0.185 | 0.670 |
| **Error** | 7554.750 | 28.000 | 269.813 | | |

26B: Attention During Saline Administration, Between-Subjects Effects, Omissions

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Rat Strain** | 225.781 | 1.000 | 225.781 | 0.562 | 0.460 |
| **Stress** | 520.031 | 1.000 | 520.031 | 1.295 | 0.265 |
| **Rat Strain * Stress** | 42.781 | 1.000 | 42.781 | 0.107 | 0.747 |
| **Error** | 11240.625 | 28.000 | 401.451 | | |

27A: Correct Responses Across Saline Day and Drug Days 1, 2, and 3, Mixed Model Analysis

| Source | Numerator df | Denominator df | F | Sig. |
|---|---|---|---|---|
| **Stress** | 1.000 | 27.812 | 4.282 | 0.048 |
| **DrugDay** | 3.000 | 41.730 | 2.012 | 0.127 |
| **Rat Strain * Stress** | 1.000 | 27.812 | 0.079 | 0.781 |
| **Rat Strain * DrugDay** | 3.000 | 41.730 | 7.347 | 0.000 |
| **Stress * DrugDay** | 3.000 | 41.730 | 0.795 | 0.504 |
| **Rat Strain * Stress * DrugDay** | 3.000 | 41.730 | 4.002 | 0.014 |

28A: Omissions Across Saline Day and Drug Days 1, 2, and 3, Mixed Model Analysis

| Source | Numerator df | Denominator df | F | Sig. |
|---|---|---|---|---|
| **Rat Strain** | 1.000 | 26.938 | 6.579 | 0.016 |
| **Stress** | 1.000 | 26.938 | 4.996 | 0.034 |
| **DrugDay** | 3.000 | 41.744 | 2.812 | 0.051 |
| **Rat Strain * Stress** | 1.000 | 26.938 | 0.528 | 0.474 |
| **Rat Strain * DrugDay** | 3.000 | 41.744 | 6.596 | 0.001 |
| **Stress * DrugDay** | 3.000 | 41.744 | 1.424 | 0.249 |
| **Rat Strain * Stress * DrugDay** | 3.000 | 41.744 | 3.244 | 0.031 |